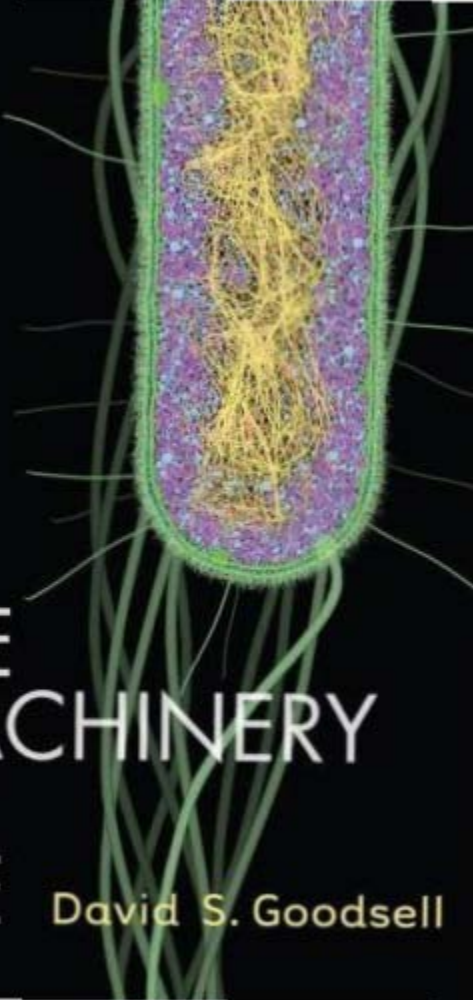




THE MACHINERY OF LIFE

David S. Goodsell

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Second Edition

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Copernicus Books

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The Immune System Piercing a Bacterial Cell Wall Our blood contains proteins that recognize and destroy invading cells and viruses. This illustration shows a cross section through a bacterial cell (lower half, in greens, blues and purples) being attacked by proteins in the blood serum (at the top, in yellows and oranges). Y-shaped antibodies begin the process by binding to the surface of the cell, and are in turn recognized by the six-armed protein at upper center. This begins a cascade of actions that ultimately lead to the formation of a membrane attack complex, shown here piercing the cell wall of the bacterium (1,000,000 X)

Preface

Imagine that we had some way to look directly at the molecules in a living organism. An x-ray microscope would do the trick, or since we're dreaming, perhaps an Asimov-style nanosubmarine (unfortunately, neither is currently feasible). Think of the wonders we could witness firsthand: antibodies attacking a virus, electrical signals racing down nerve fibers, proteins building new strands of DNA. Many of the questions puzzling the current cadre of scientists would be answered at a glance. But the nanoscale world of molecules is separated from our everyday world of experience by a daunting million-fold difference in size, so the world of molecules is completely invisible.

I created the illustrations in this book to help bridge this gulf and allow us to see the molecular structure of cells, if not directly, then in an artistic rendition. I have included two types of illustrations with this goal in mind: watercolor paintings which magnify a small portion of a living cell by one million times, showing the arrangement of molecules inside, and computer-generated pictures, which show the atomic details of individual molecules. In this second edition of *The Machinery of Life*, these illustrations are presented in full color, and they incorporate many of the exciting scientific advances of the 15 years since the first edition.

As with the first edition, I have used several themes to tie the pictures together. One is that of scale. Most of us do not have a good concept of the relative sizes of water molecules, proteins, ribosomes, bacteria, and people. To assist with this understanding, I have drawn the illustrations at a few consistent magnifications. The views showing the interiors of living cells, as in the Frontispiece and scattered through the last half of the book, are all drawn at one million times magnification. Because of this consistent scale, you can flip between pages in these chapters and compare the sizes of DNA, lipid membranes, nuclear pores, and all of the other molecular machinery of living cells. The computer-generated figures of individual molecules are also drawn at a few consistent scales to allow easy comparison.

I have also drawn the illustrations using a consistent style, again to allow easy comparison. A space-filling representation that shows each atom as a sphere is used for all the illustrations of molecules. The shapes of the

molecules in the cellular pictures are simplified versions of these space-filling pictures, capturing the overall form of the molecule without showing the location of every atom. The colors, of course, are completely arbitrary since most of these molecules are colorless. I have chosen them to highlight the functional features of the molecules and cellular environments.

In the drawings of cellular interiors, I have made every effort to include the proper number of molecules, in the proper place, and having the proper size and shape. In the 15 years since publication of the first edition, a remarkable variety of new data have become available to support these pictures, but the published data on the distribution and concentration of the molecules are still far from complete. As a result, the cellular pictures are subject to some personal interpretation, especially the illustrations of human cells in Chapters 5 and 6.

As in the first edition, I have written the text with the nonscientist reader in mind, and I have drawn the illustrations at a level of scientific rigor meant to satisfy readers who are scientists. For the lay reader, this book is an introduction to molecular biology—a pictorial overview of the molecules that orchestrate the processes of life. The new edition includes many new results from the study of molecular biology, and includes a new chapter on life, aging and death.

Please note, however, that this book is not meant to be comprehensive—I have chosen a series of subjects that capture the aspects of molecular biology that I find most salient, and most fascinating. The reader is referred to the excellent textbooks listed at the end of the book for more detailed and comprehensive information. In particular, *Molecular Biology of the Cell* will point the way to further study of nearly any subject in cell or molecular biology. For the scientist, it is my hope that this book will continue to provide a touchstone for intuition. Please use the illustrations, as I have, to help imagine biological molecules in their proper context: packed into living cells.

I thank the people who have been instrumental in seeing this project from concept to conclusion. Arthur Olson has continued to provide useful comments at every stage, as well as providing an amazing working environment in the Molecular Graphics Laboratory at the Scripps Research Institute in La Jolla. Much of the material from this book draws on my Molecule of the Month series at the RCSB Protein Data Bank, which has kindly supported my illustration and writing for the past 8 years. I also thank the Fondation Scientifique Fourmentin-Guilbert for their kind support of this project. The computer-generated illustrations were produced with methods that I developed under the auspices of the Daymon Runyon-Walter Winchell Cancer Research Fund, the National Institutes of Health, and the National Science Foundation. Finally, I would like to thank Bill Grimm for his support and confidence.

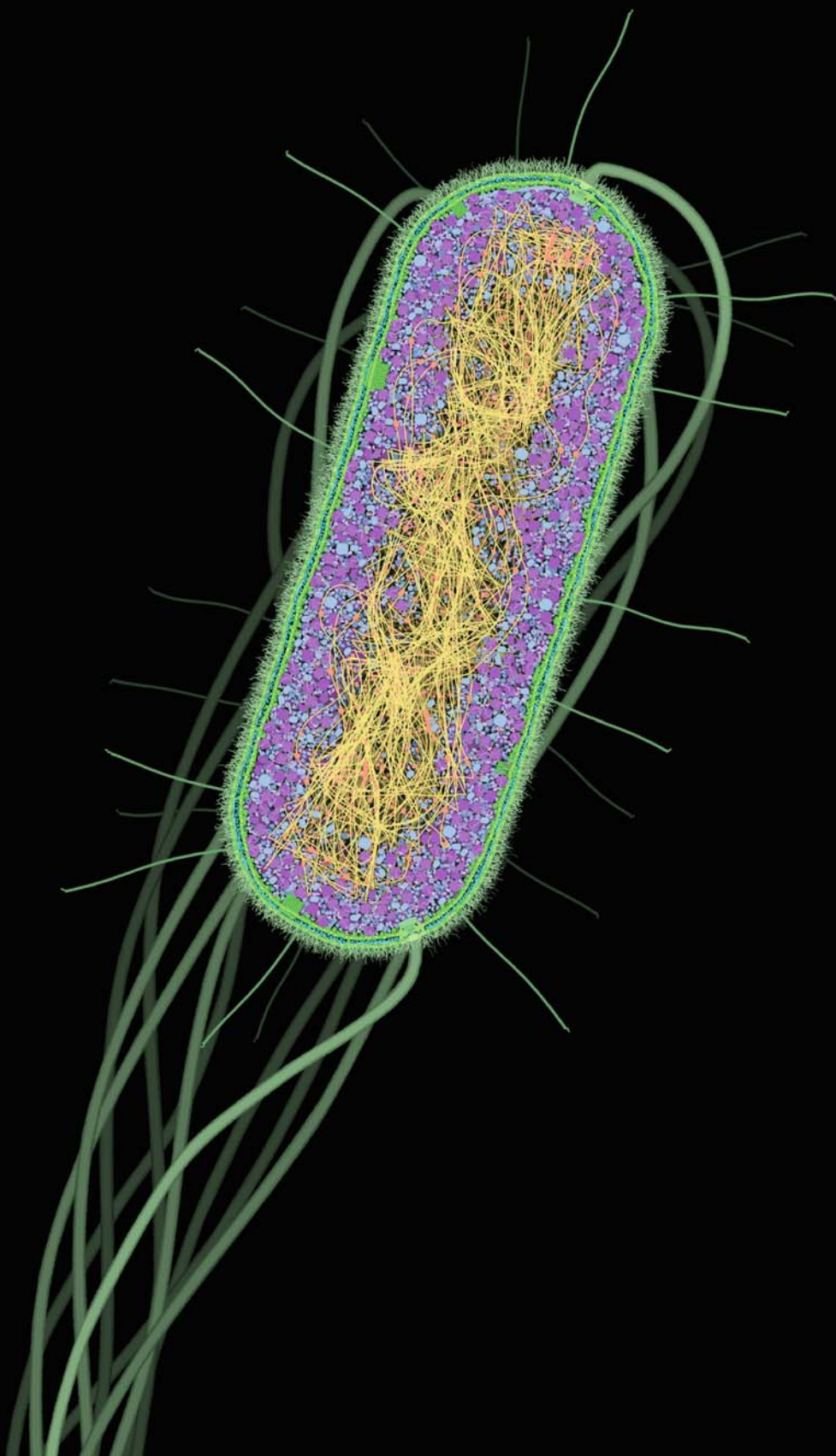
La Jolla, CA

D.S. Goodsell

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Chapter 1

Introduction

Our world is overflowing with the diversity of life. Imagine that you are taking a leisurely walk through a wooded park. Oak and maple trees cast dancing shadows under the noonday sun. Birds and butterflies dart through the air and a squirrel scampers noisily up a tree trunk. In a typical woodland area, you will be surrounded by dozens of types of trees and plants, filled with even more types of birds. Insects will be crawling on the ground, climbing in the foliage, and flying through the air. Even in the middle of the city, you can find a huge selection of different plants—some carefully tended and some stealthily avoiding the gardener—filled with a variety of birds and insects, all scraping out a living between the houses and concrete.

Next time you are wandering through a park or hiking in the woods, anywhere there are plants and animals, take a moment to examine your surroundings with the eye of a biologist. At its best, science reveals the many wonders hiding behind our familiar experience of the world . . . and this woodland scene carries the evidence of something truly remarkable. By examining the plants, birds, and animals that surround us, scientists have discovered that you and I are directly related to every other living thing on the Earth. With a little bit of critical observation, you can discover this relationship yourself.



Fig. 1.1 The Machinery of Life All life on the Earth is composed of cells, which are themselves composed of molecules. A cross section through a single bacterial cell is shown here. It is surrounded by a multi-layered cell wall, colored green. The long corkscrew-shaped flagella are turned by motors in the cell wall, propelling the cell through its environment. The interior of the cell is filled with molecular machines for building and repairing molecules, for harnessing different sources of energy, and for sensing and protecting against environmental dangers (70,000 X)

A casual look is enough to show that you are closely related to your mother and father, to your brothers and sisters, and even to the other men and women who might be hiking past you on a wooded trail or crowded boulevard. We are all negligibly different, varying only in nuances of proportion and subtleties of shade. We share the same senses, we walk and talk using the same combination of muscles and bones, we are all born the same way, and we all die as our bodies wear out after a similar amount of time. You do not need to trace family trees to prove that all human beings are related: you can tell just by looking.

The relationship to our biological next of kin, however, requires a bit more observation to discover. A trip to the zoo will reveal our close kinship with familiar animals. Birds and mammals, reptiles, amphibians, and fish are all cousins many times removed. A short study of anatomy is needed to show the family resemblance. We all share similar digestive and nervous systems and a system of bones and muscles built around a head, torso, and four limbs. The differences between us and elephants or lizards are trifles of magnitude: longer legs, denser fur, or sharper teeth.

Things get really interesting when you start looking at our extended biological family. This includes all living things: plants, sponges, insects, flatworms, and all sorts of exotic distant relatives. You need to use many of the tools of biology to see our relationship with these family members. A study of anatomy does not help much—you are so different from a tree that it is difficult to make meaningful analogies between, for instance, your stomach and tree roots, both of which are used to gather food. But when you look in the microscope, you will find that all living organisms are composed of cells, and that the cells in a tree look amazingly similar to the cells in your own hand.

Perhaps the most remarkable observation from biology is that even bacteria share this family history with us. Bacteria are composed of a single cell (Fig. 1.1) instead of the trillions of cells that make up your own body, but that single cell uses much the same machinery as your own cells. If you look very closely at the molecules that orchestrate the processes of life, the resemblance is apparent (Fig. 1.2). Every living thing on Earth uses a similar set of molecules to eat, to breathe, to move, and to reproduce. Because of this, trees and frogs and botulism bacteria all require water and food, they all will die if they get too hot or too cold, and they can reproduce and make new trees and frogs and botulism bacteria if the conditions are just right.

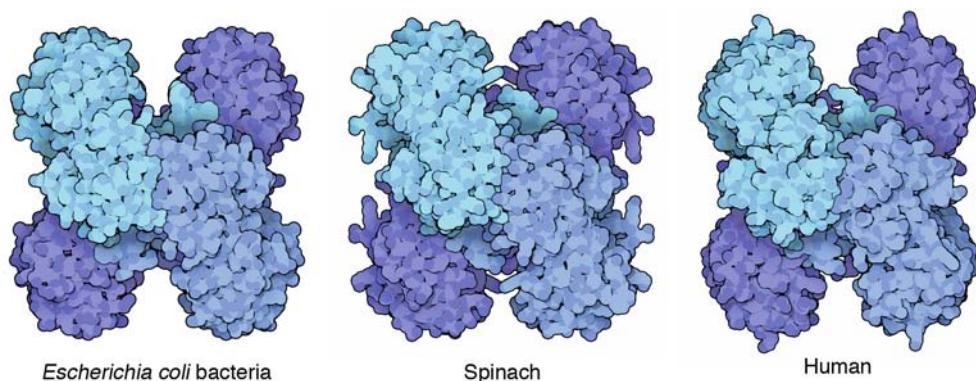


Fig. 1.2 Molecular Machinery Many molecular machines are virtually identical in all living cells. This is particularly true for molecules that play an essential role in the processes of life, such as the enzyme glyceraldehyde-3-phosphate dehydrogenase, which is vital for the metabolism of sugar in all three organisms. This illustration shows the similar form of the enzyme from a bacterial cell (left), a plant cell (center), and human cells (right) (5,000,000 X)

In this book we will explore this common birthright of molecular machines. We will start with a look at the machines themselves and the unusual molecular world in which they operate. Then, we will explore how they are combined in living cells. Finally, we will look at a few special topics related to our own molecules and cells.

A Matter of Scale

Almost everything that we will discuss in this book is too small to see. Cells are small but not unimaginably small, and molecules are really, really small. Cells are about 1000 times smaller in length than objects in our everyday world. The largest cells, such as protozoa, can be seen with a magnifying glass, but a microscope is needed to see most of the cells in your body. Typical human cells are about 10 μm in length. This is roughly 1000 times smaller than the last joint in your finger. A 1000-fold difference in size is not difficult to visualize: a grain of rice is about 1000 times smaller in length than the room you are sitting in. Imagine your room filled with grains of rice. That will give you an idea of the billion or so cells that make up your fingertip.

Another 1000 times reduction takes us to the world of molecules. Molecules are so small that they are smaller than the wavelength of

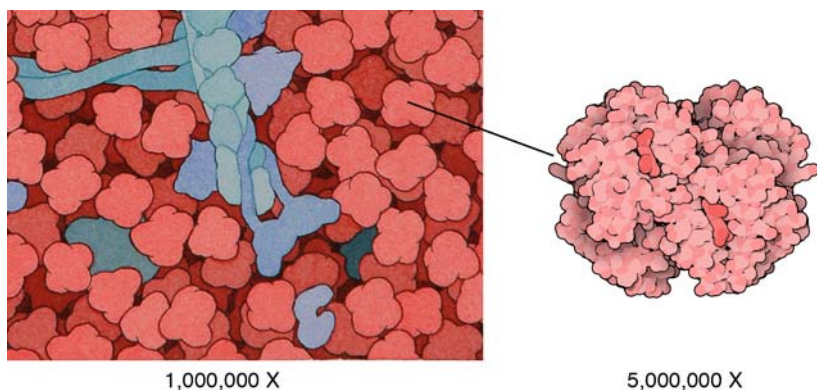


Fig. 1.3 Molecular Illustrations Two types of pictures are used for most of the illustrations in this book. To create pictures of individual molecules, such as the hemoglobin molecule shown on the right here, a computer program draws a sphere for each atom in the molecule, centered on the nucleus of the atom and approximately the size of the cloud of electrons that surrounds the nucleus. In these illustrations, you can easily see individual atoms—this one is drawn at 5,000,000 X magnification. In the hand-drawn illustrations that show molecules inside cells, such as the portion of a red blood cell shown on the left, the shapes of these molecules are simplified, and individual atoms are too small to be seen (they would be about the size of a grain of salt at this magnification). The hand-drawn illustrations are presented at a consistent magnification of 1,000,000 X

light, so there is no way to “see” them directly with a light microscope. Instead, we use methods like x-ray crystallography, NMR spectroscopy, electron microscopy, or atomic force microscopy to discover the arrangement of atoms in the molecule, and then we create artificial pictures of them (Fig. 1.3). An average protein, taken from any cell, contains about 5000 atoms and is about one-thousandth the length of a typical cell, or about one-millionth the width of your fingertip. Again, to get an idea of these sizes, think of a room filled with rice grains. This will give an idea of the size of the proteins that are packed into each of your cells.

The Molecular World

The molecules in our cells perform their jobs in a strange, unfamiliar world, so we have to be careful. When trying to understand a molecular process, our intuition may lead us astray. The principles that guide objects in our everyday world—gravity, friction, temperature—

are different at the molecular scale and often have surprisingly different effects.

One basic thing remains the same at our size and at molecular size: the solidity of matter. At the scale of molecules, we do not need to worry too much about the odd things that happen with quantum mechanics: to a first approximation, molecules have a definite size and shape, and it is perfectly fine to imagine them bumping into each other and fitting together if the shapes match. If we look closely, their edges may be a bit fuzzy, but for most purposes, we can think of them as physical objects like tables and chairs.

Other properties, however, are very different when we enter the molecular world. For instance, molecules are so small that gravity is completely negligible. The motions and the interactions of biological molecules are completely dominated by the surrounding water molecules. At room temperature, a medium-sized protein travels at a rate of about 5 m/s (the speed of a fast runner). If placed alone in space, this protein would travel its own length in about a nanosecond (a billionth of a second). Inside the cell, however, this protein is battered from all sides by water molecules. It bounces back and forth, always at great speed, but takes a long time to get anywhere (Fig. 1.4). When

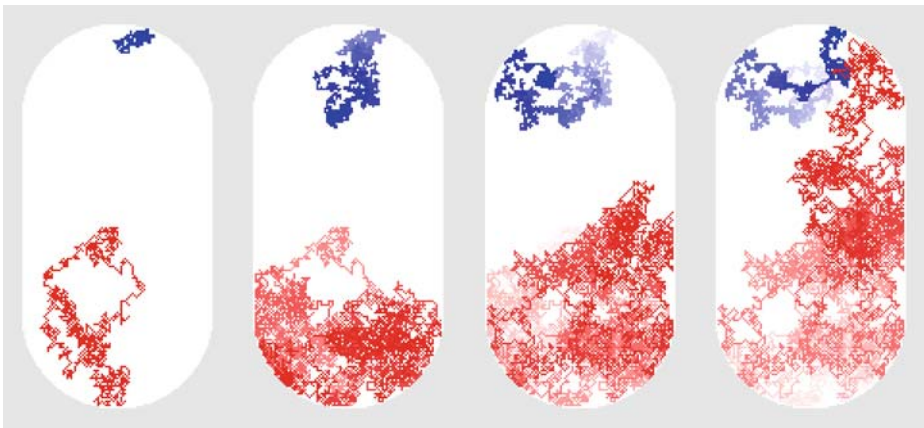


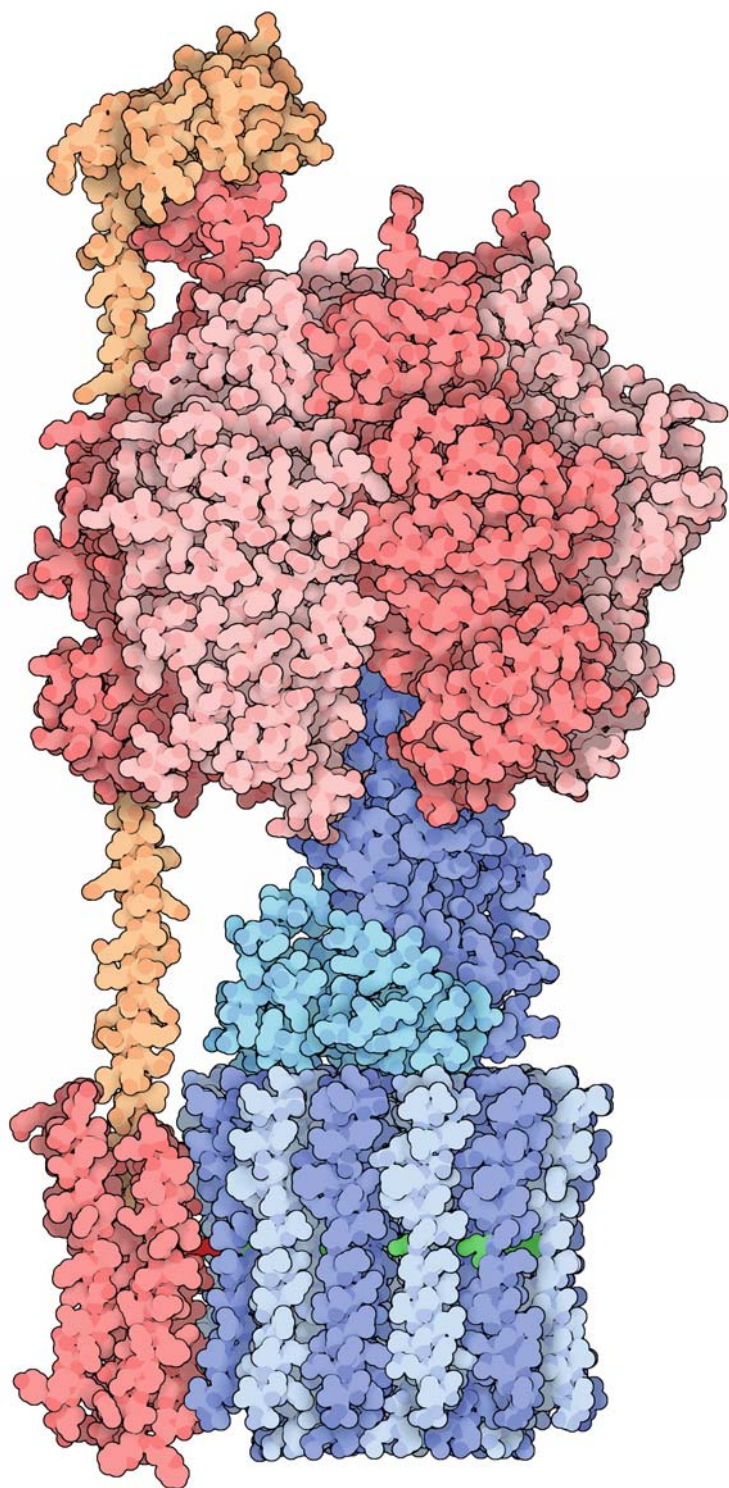
Fig. 1.4 Molecular Diffusion Molecules constantly diffuse within the interior of cells, randomly bumping from place to place. This illustration shows several snapshots from a computer simulation of a protein and a sugar molecule diffusing inside a bacterial cell. The path of the protein is shown in blue and the path of sugar is shown in red. They start at opposite ends and explore much of the space inside before they reach each other

surrounded by water, this typical protein now requires almost a thousand times longer to move a protein-sized distance.

Imagine a similar situation in our world. You enter an airline terminal and want to reach a ticket window on the far side of the room. The distance is several meters—a distance comparable to your own size. If the room is empty, you dash across in a matter of seconds. But imagine instead that the room is crowded full of other people trying to get to their respective windows. With all the pushing and shoving, it now takes you 15 minutes to cross the room! In this time, you may be pushed all over the room, perhaps even back to your starting point a few times. This is similar (although molecules do not have a goal in mind) to the contorted path molecules take in the cell.

You might ask how anything ever gets done in this chaotic world. It is true that the motion is random, but it is also true that the motion is very fast compared to the motion in our familiar world. Random, diffusive motion is fast enough to perform most of the tasks in the cell. Each molecule simply bumps around until it finds the right place.

To get an idea of how fast this motion is, imagine a typical bacterial cell like the one in Fig. 1.1, and place an enzyme at one end and a sugar molecule at the other. They will bump around and wander through the whole cell, encountering many molecules along the way. On average, though, it will only take about a second for those two molecules to bump into each other at least once. This is truly remarkable: this means that any molecule in a typical bacterial cell, during its chaotic journey through the cell, will encounter almost every other molecule in a matter of seconds. So as you are looking at the illustrations in this book, remember that static images give only a single snapshot of this teeming molecular world.



Chapter 2

Molecular Machines

The human body is a living, breathing example of the power of nanotechnology. Almost everything happens at the atomic level. Individual molecules are captured and sorted, and individual atoms in these molecules are shuffled from place to place, building entirely new molecules. Individual photons of light are captured and used to direct the motion of individual electrons through electrical circuits. Molecules are packaged and transported expertly over distances of a few nanometers. Tiny molecular machines, such as the one in Fig. 2.1, orchestrate all of these nanoscale processes of life. Like the machines of our modern world, these machines are built to perform specific tasks efficiently and accurately. These tasks, however, are molecule-sized tasks and the molecular machines in cells have been perfected to operate at the level of atoms.

As we will see throughout this book, molecular machines have many similarities with familiar machines like scissors and automobiles. The unusual, organic shapes of molecular machines may seem daunting and incomprehensible, but in many ways, molecular machines may be understood in a similar way: as a mechanism where parts fit together, move, and interact to perform a given job. Molecular machines, however, have a few fundamental differences from man-made machines, and it is necessary to gain a basic understanding of these differences to appreciate the wonders occurring at the molecular scale.

One major challenge is that molecular machines must be made of atoms. This may seem obvious, but it actually causes deeply rooted



Fig. 2.1 ATP Synthase ATP synthase is a molecular machine used in the production of chemical energy. It is constructed from over 40,000 atoms, each in a specific place and performing a specific function (8,000,000 X)

problems. Atoms only come in a few shapes and sizes. Cells do almost all of their work with six types of atoms—carbon, oxygen, nitrogen, sulfur, phosphorous, and hydrogen—adding more exotic atoms only when needed for special tasks. These atoms may be connected in only very limited ways, defined by the underlying chemistry of the atoms. Molecular machines must be constructed within these significant limitations. It is much like trying to build machines with Tinkertoys or Lego blocks: you may build a wide variety of different objects, but the final form is shaped and limited by the shapes and connections of the underlying units. We will see that molecular machines take advantage of every trick to make best use of their limited set of raw materials.

Modern cells use four basic plans for combining atoms to make molecular machines. Whereas our familiar machines are built of metal, wood, plastic, and ceramic, the nanoscale machinery of cells is built of protein, nucleic acid, lipid, and polysaccharide. Each of these plans has a unique chemical personality ideally suited to a different role in the cell. Two basic concepts are needed to understand how this chemical personality is manifested: *chemical complementarity* and *hydrophobicity*.

When molecules come into contact, they interact with one another. In most cases, the interaction is not strong, so they just bump into each other and then go on their way. However, if the interaction is complementary, they bind tightly to one another. Molecules interact through a number of specific atom-to-atom interactions. Most are individually weak, but they add up and become important when a large portion of one molecule fits perfectly next to a similarly shaped portion of a neighboring molecule (Fig. 2.2). Molecular machines also take advantage of two types of specific interactions: hydrogen bonds between a hydrogen atom and an oxygen or nitrogen atom, and salt bridges between atoms that carry opposite electrical charges. These specific interactions act like little fasteners to lock molecules together.

Hydrophobicity is a more elusive concept that is caused by the unusual properties of water. Molecules tend to interact in one of two ways with water. On one hand, molecules that interact strongly with water, often molecules rich in oxygen and nitrogen atoms, are termed *hydrophilic* (“water loving”). Hydrophilic molecules dissolve easily in water, surrounding themselves with a comfortable shell of water molecules. Table sugar and acetic acid (the sour acid in vinegar) are familiar hydrophilic small molecules. On the other hand, molecules that are rich in carbon atoms do not interact well with water, and are termed *hydrophobic* (“water fearing”). When placed in water, these molecules

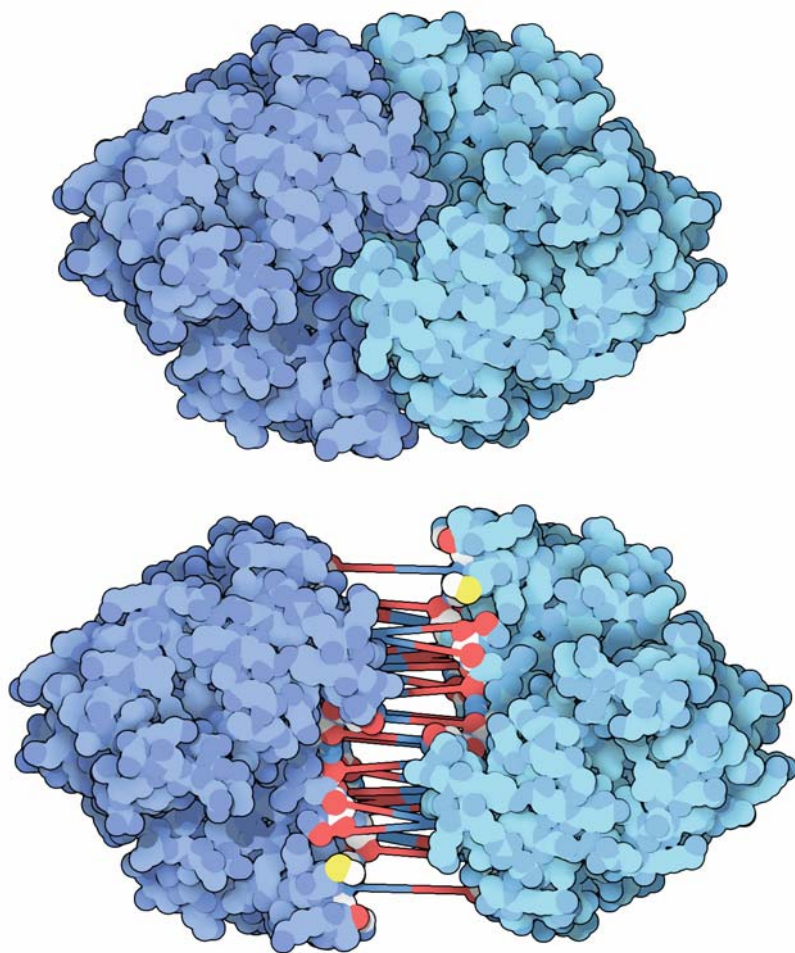


Fig. 2.2 Chemical Complementarity Biological molecules interact through large, complementary patches on their surfaces. The enzyme enolase, which performs one step in the metabolism of sugar, is shown here. It is composed of two subunits that combine to form the active molecular machine. The image at the bottom shows the two subunits separated, with lines connecting atoms that form hydrogen bonds. Notice how the two shapes interlock and match perfectly when they come together (10,000,000 X)

tend to cluster together, gathering into globules away from the surrounding water (Fig. 2.3). This is what happens with vegetable oil in water: droplets form to minimize the contact of hydrophobic oil molecules with water.

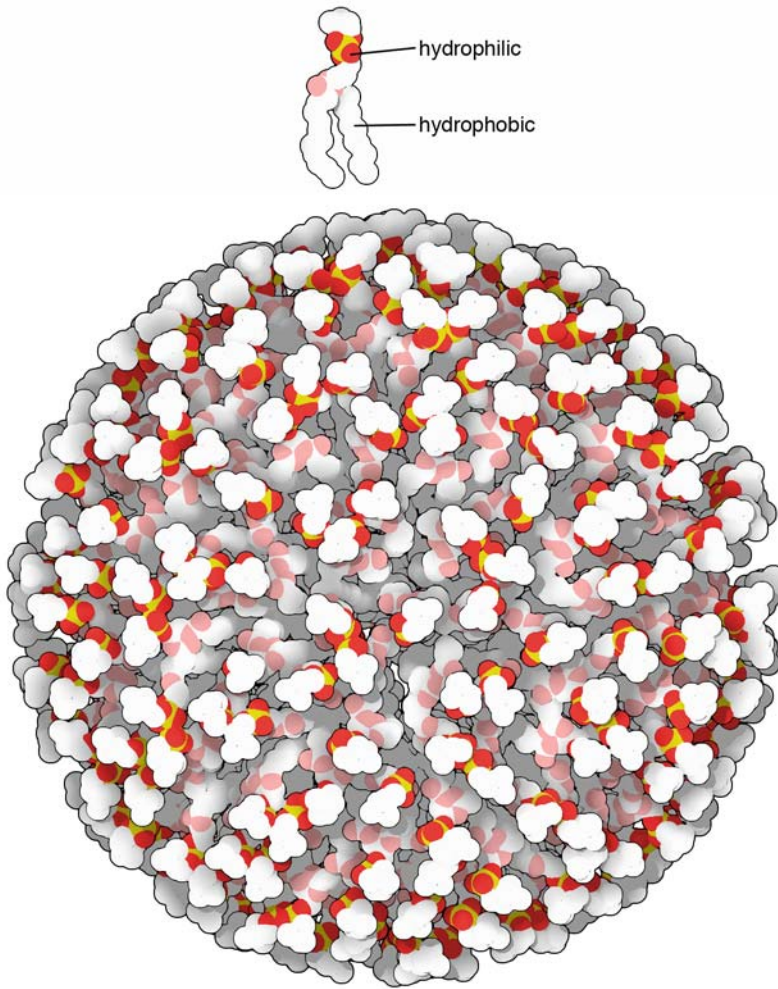


Fig. 2.3 Hydrophobicity The phospholipid shown at the top has a phosphate group, shown in bright yellow and red, which is hydrophilic—it interacts strongly with water. The rest of the molecule is composed primarily of carbon and hydrogen (colored white), which is hydrophobic and does not interact strongly with water. When lipids are mixed with water, they separate into small droplets (or lipid bilayers, as described later in the chapter), minimizing the contact with the surrounding water. At the bottom, many phospholipids have coalesced into a compact globule, with all the hydrophobic parts sheltered inside

The large molecular machines in cells take advantage of both of these properties. They often have oddly shaped surfaces that use hydrogen bonds and salt bridges to find molecules with complementary shapes. They often have both hydrophilic and hydrophobic regions that interact differently with water. Different patterns of these regions cause molecules to act in novel ways when dissolved in water. The four molecular plans—proteins, nucleic acids, lipids, and polysaccharides—use different combinations of these properties to achieve different molecular goals.

Nucleic Acids

Nucleic acids specialize in the use of chemical complementarity to encode information. They play an essential role—some would say the central role—in the processes of living. Nucleic acids store and transmit the genome, the hereditary information needed to keep a cell alive. All of the information describing how to make proteins, and when to make them, is stored in strands of nucleic acid at the center of each cell.

The unique interactions between nucleic acid chains make them ideally suited to this role as the cell's librarians. Nucleic acids are composed of long chains of nucleotides, each of which has a specific arrangement of hydrogen-bond-forming atoms. These atoms are the reason that nucleic acids are so useful. In DNA (deoxyribonucleic acid), these nucleotides come in four varieties—adenine (A), thymine (T), cytosine (C), and guanine (G)—and these four nucleotides match perfectly in one special combination—matching A with T and C with G—but not in any of the other ways.

This specific matching is the basis for ability of nucleic acids to store and transmit information. Just like the strings of numbers in a computer disk, information may be stored in the sequence of nucleotides along a nucleic acid strand. For instance, the sequence A-T-G is a universal code that means "Start." This information may be read using specific hydrogen bonds: a new strand may be built by matching up nucleotides one-by-one along the strand—A with T and C with G—and then connecting the new nucleotides together. The result is a unique new strand with complementary information content. This strand can then be used to build another, and another, and so on, generation after generation.

The chemical structure of nucleotides is perfect for assisting this transfer of information (Figs. 2.4 and 2.5). Each nucleotide is composed of a base, which includes the hydrogen-bonding atoms held together in the perfect orientation in a rigid ring, and a

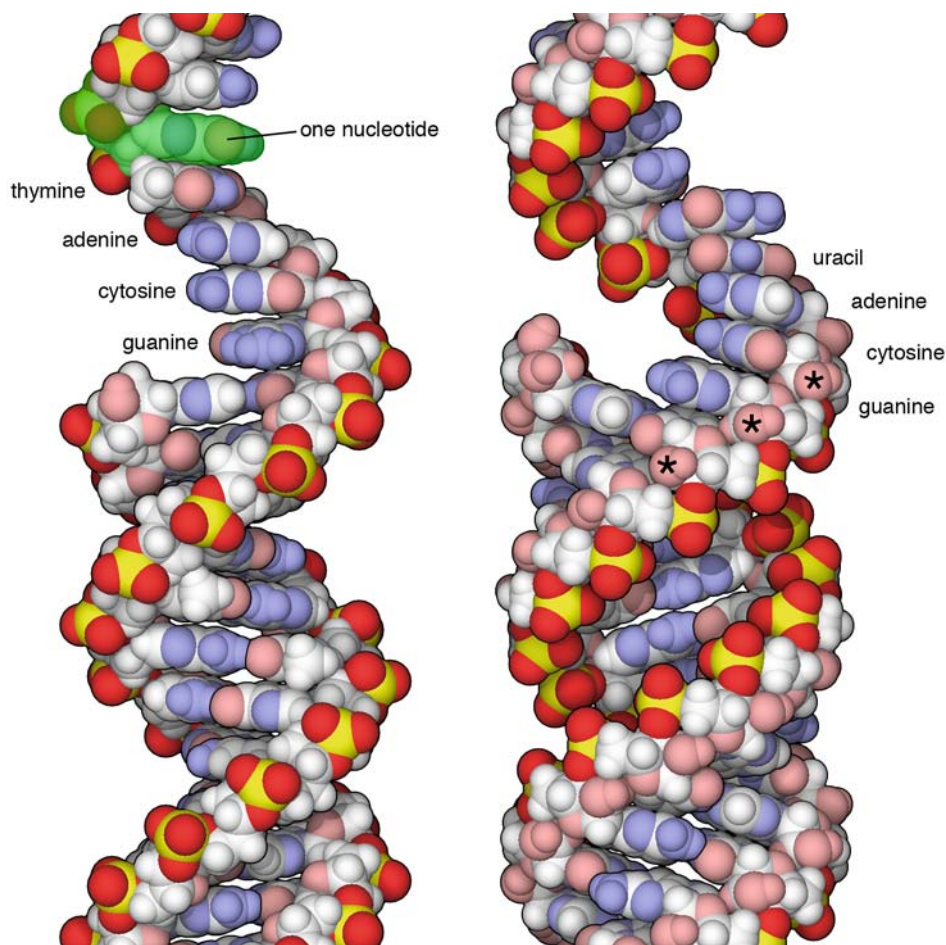


Fig. 2.4 Nucleic Acid Structure Nucleic acids are composed of long chains of nucleotides. In this illustration, hydrophobic carbon atoms are in white, mildly hydrophilic atoms are in pastel shades (light blue for nitrogen and pink for oxygen), and strongly charged hydrophilic atoms are in bright colors (blue for nitrogen, red for oxygen, and yellow for phosphorus). Hydrogen atoms, which are shown with smaller spheres, are colored the same as the atom to which they are bonded. DNA is shown on the left and RNA is shown on the right, with a double helix at the bottom and a single strand at the top. The asterisks on the RNA strand show a few of the oxygen atoms that are present in RNA, but not in DNA (20,000,000 X)

sugar-phosphate group, which is used to link the nucleotides together. The sugar-phosphate groups are relatively flexible, so the chain can bend into many functional shapes, and the highly charged phosphates make the chain very soluble in water. The bases, on the other hand, are

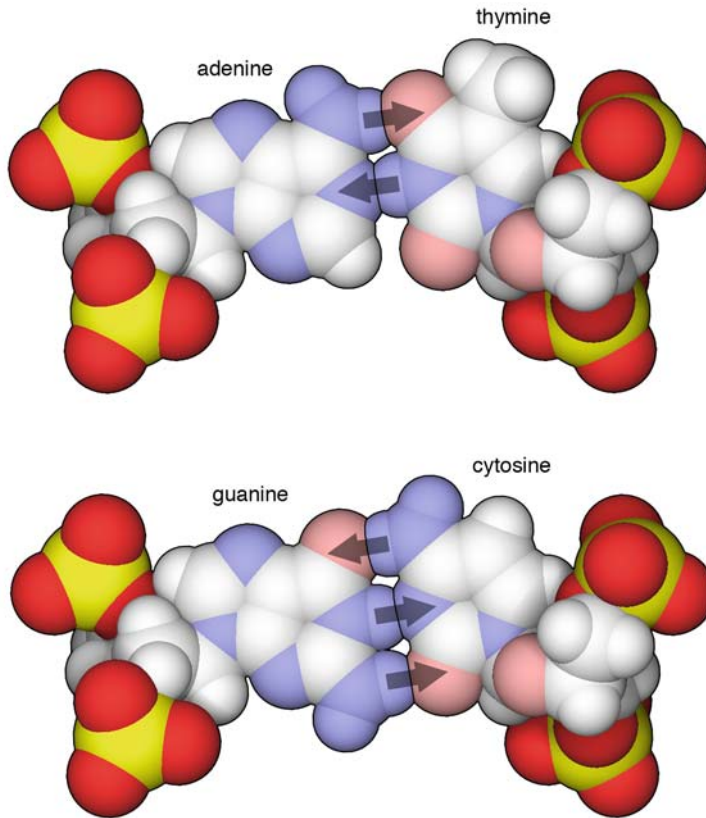


Fig. 2.5 Information Transfer in Nucleic Acids DNA bases interact through a series of specific hydrogen bonds, matching adenine with thymine and cytosine with guanine. The hydrogen bond interaction is strongest when the bases are aligned perfectly side-by-side, matching a hydrogen atom on one base with an oxygen or nitrogen on the complementary base. Adenine and thymine form two of these hydrogen bonds (shown with arrows), and guanine and cytosine form three (40,000,000 X)

rather hydrophobic and favor shapes where they are stacked on top of one another, sheltered away from water. This is why DNA forms the familiar double helix: two strands line up with all the bases stacked on the inside and all the phosphate groups winding around on the outside.

Nucleic acids are built in two varieties in cells: DNA and RNA (Fig. 2.6). RNA (ribonucleic acid) differs chemically from DNA in two

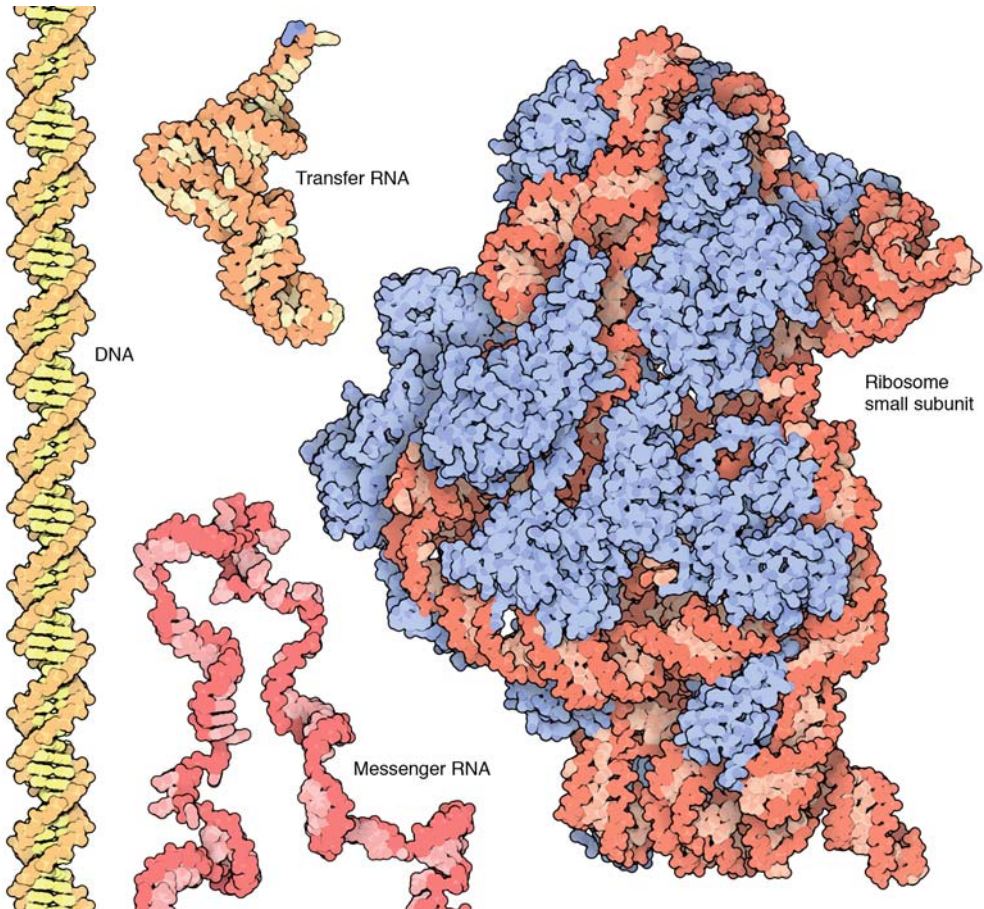


Fig. 2.6 Nucleic Acid Functions Nucleic acids perform several tasks in cells. The DNA double helix is the primary storehouse of genetic information, and long messenger RNA strands are temporary carriers of this information. Transfer RNA and ribosomes (which are composed of RNA and protein) are essential machinery for the synthesis of proteins (5,000,000 X)

small ways: the sugar has an extra oxygen atom and the thymine base is replaced by uracil, which is smaller by a carbon atom and a few hydrogens. These small chemical differences actually make a big difference in the function of RNA. The extra oxygen atom makes RNA a bit less stable than DNA, so DNA is primarily used as the central storehouse of information and RNA is used for temporary information processing. Disposable strands of RNA are copied from DNA

templates, clipped, capped, and edited, transported around the cell to perform their functions, and finally discarded when the job is done.

Nucleic acids, however, are too limited in structure to perform the many tasks in the daily life of a cell. The chemistry of the four bases, although perfect for information transfer, are too similar to allow the construction of the varied machines needed to perform thousands of different chemical and mechanical reactions. Instead, proteins are used in this capacity.

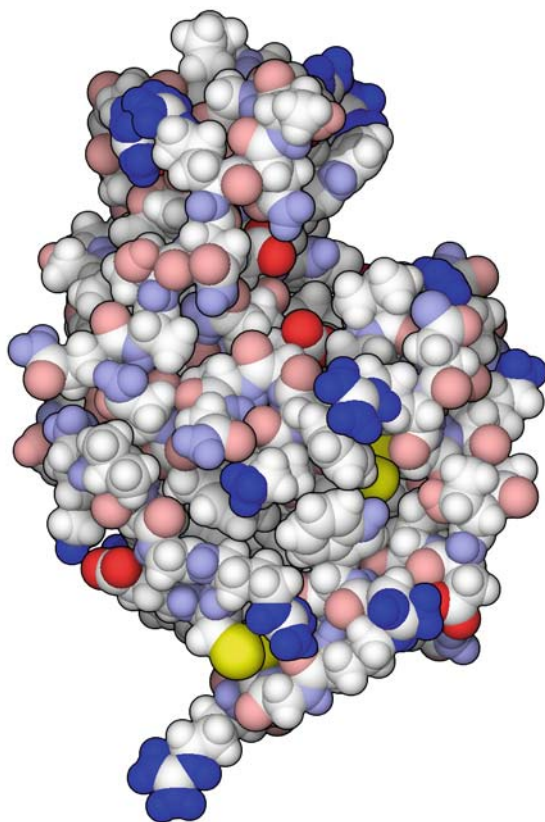
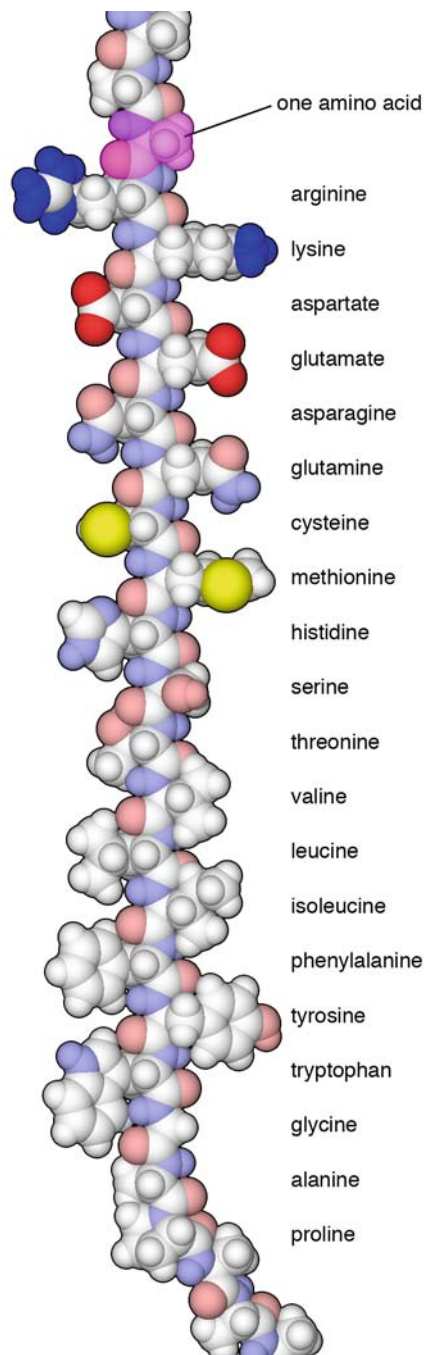
Proteins

Look anywhere in a cell and you will see proteins at work. Proteins are built in thousands of shapes and sizes, each performing a different molecule-sized function. Some are built simply to adopt a defined shape, assembling into rods, nets, hollow spheres, and tubes. Some are molecular motors, using energy to rotate or flex or crawl. Many are chemical catalysts that perform chemical reactions atom-by-atom, transferring and transforming chemical groups exactly as needed.

Proteins have a modular chemical structure that allows the construction of widely different molecular machines using the same basic set of building blocks. Like nucleic acids, proteins are long molecular chains, but instead of using four chemically similar nucleotides, proteins are built of 20 different amino acids, each with a different size and chemical character (Fig. 2.7). Some amino acids carry an electrical charge and interact strongly with water and ions. Others are composed mostly of carbon and hydrogen atoms and are strongly hydrophobic. Some are big and bulky; others are small and can fit into tight corners. Some are rigid and others very flexible. Some are chemically reactive and others are completely neutral. By using this diverse alphabet of amino acids, cells are able to build an even more diverse vocabulary of proteins.

When protein chains are placed in water, they perform a remarkable feat. They twist and fold, finding an optimal shape that will shelter the hydrophobic amino acids inside and display the charged amino acids on the surface. Amazingly, proteins are self-assembling machines. This process happens all by itself, or with a little assistance from chaperone proteins that protect the folding chain from the meddling influence of neighboring proteins. The final shape of the folded protein is completely predetermined by the order of amino acids in the protein chain.

As you might imagine, only a small fraction of the possible combinations of amino acids will fold spontaneously into a stable structure. If you make a protein with a random sequence of amino acids, chances



are that it will only form a gooey random tangle when placed in water. Cells have perfected the sequences of amino acids in their proteins over many years of evolutionary selection, and scientists are only now discovering the tricky rules that guide the process of folding, allowing us to design our own proteins.

Proteins have unusual, organic shapes, quite unlike the machines in our familiar world (Fig. 2.8). With their wide potential for diversity, proteins are constructed to perform most of the everyday tasks of the cell. A typical bacterium builds several thousand different types of proteins, each with a different function. Our own cells build about 30,000 different kinds, ranging in size from small protein hormones like glucagon, which has only 29 amino acids, to huge proteins like titin, which has over 34,000 amino acids. Nearly every job in the cell is performed by a protein, except in special cases where the unique chemical natures of nucleic acids, lipids, and carbohydrates are needed. Proteins are the jacks-of-all-trades, pressed to duty in innumerable shapes and forms.

Lipids

Individually, lipids are tiny molecules, but when grouped together, they form the largest structures of the cell. When placed in water, lipid molecules aggregate to form huge waterproof sheets. These sheets are used to enclose cells, forming the primary boundary separating the inside of the cell from the environment. They are also used to build compartments inside cells, such as the nucleus and the mitochondria.

The unusual interaction of lipids with water makes them so useful. Lipids, known commonly as fats and oils, are composed of a small hydrophilic “head” connected to two or three long hydrophobic “tails.” When placed in water, lipid molecules spontaneously aggregate, packing molecules side-by-side to shelter the long tails away from



Fig. 2.7 Protein Structure Proteins are composed of a chain of amino acids that folds into a compact globular structure. All 20 amino acids are shown in the extended chain on the left. Notice the different shape and chemical composition of each one. The four at the top carry a strong electric charge, the ones in the middle are slightly charged, and the ones at the bottom are hydrophobic. The last one, proline, forms a rigid kink in the protein chain. The small protein lysozyme, which contains 129 amino acids, is shown on the right (20,000,000 X)

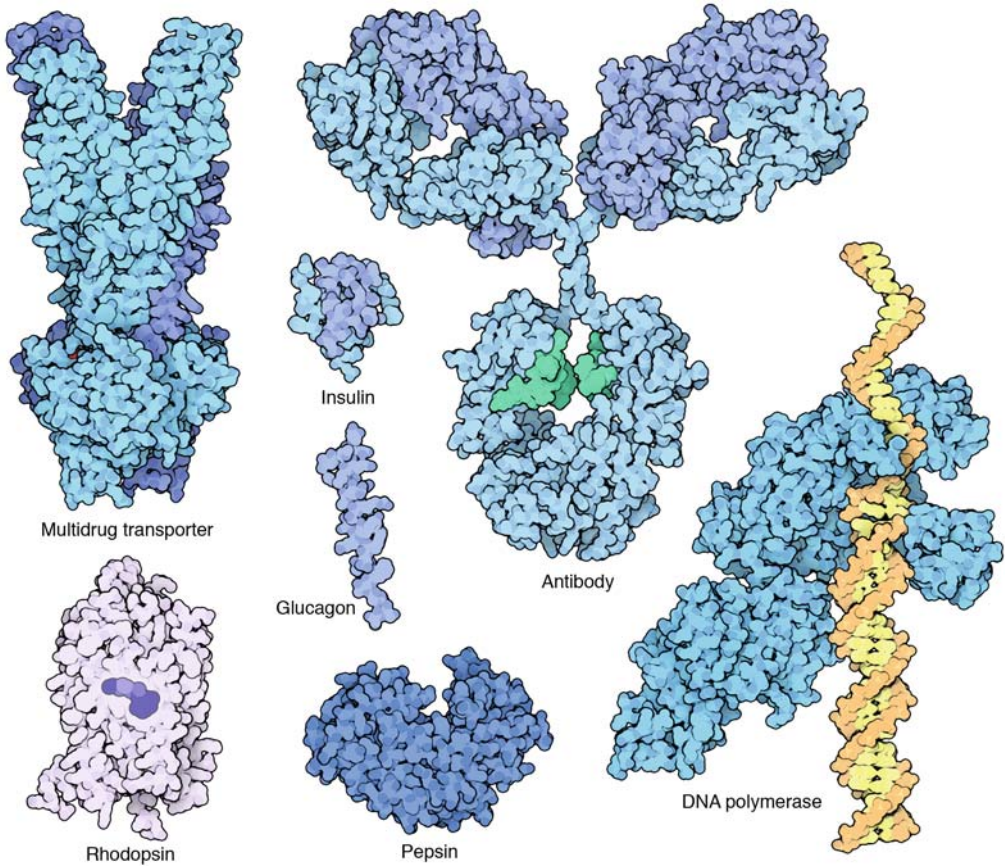
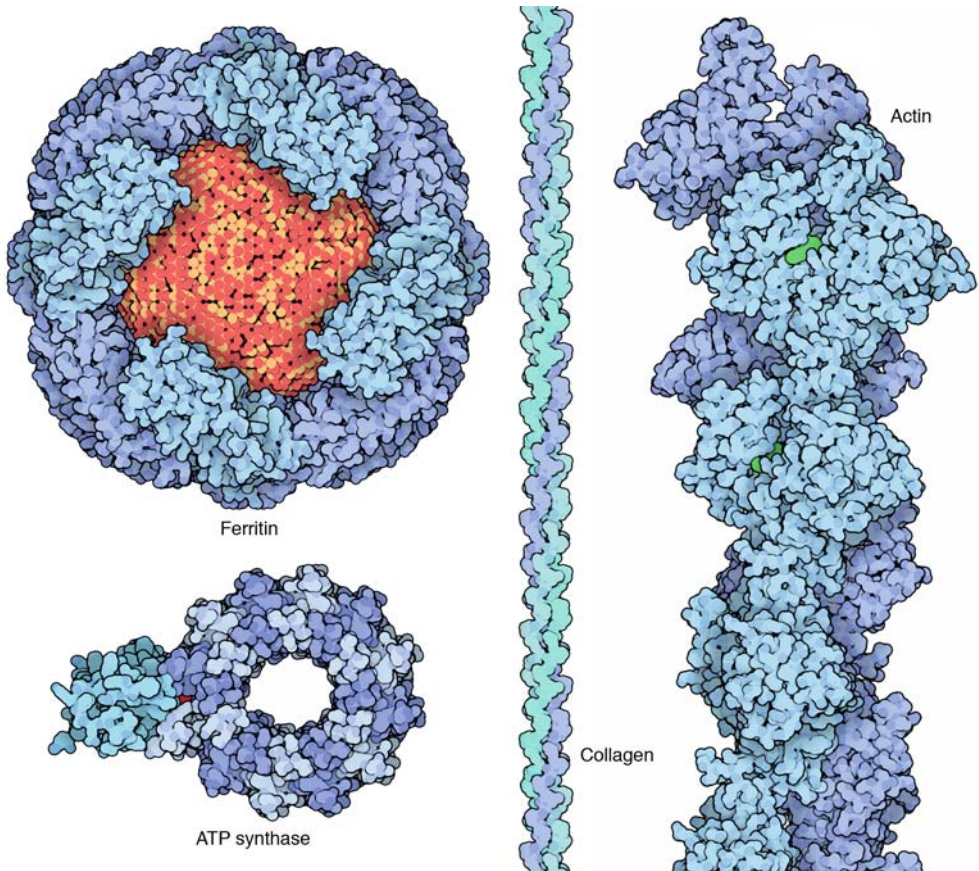


Fig. 2.8 Protein Functions Proteins are constructed for many different functions. In many cases, several protein chains fold, and then associate to form a larger structure. The multidrug transporter pumps drugs and poisons out of cells using a scissor-type motion of its two chains. Rhodopsin is the sensor of light in our retinas—it uses a colorful molecule of retinal to capture light. Insulin and glucagon are hormones that deliver opposite messages about blood sugar levels. Pepsin is the protein-digesting machine that breaks down food in the stomach—because of this function, it must be highly resistant to acid. Antibodies specialize in recognition of foreign objects—they find viruses and bacteria in the blood during



infections. DNA polymerase copies the information held in a DNA strand—this one is from a bacterium that lives in hot springs, so it is very stable and is used in biotechnology to copy DNA. Ferritin stores iron ions inside cells—it is a protein shell that surrounds a small crystalline mass of about 4000 iron ions and hydroxide ions. This part of ATP synthase is a rotary motor powered by electrochemical energy. Collagen forms long, sturdy cables that support our organs and tissues, and is the most common protein in the human body. Actin also forms structural girders, but ones that may be assembled and disassembled at will (5,000,000 X)

the water. You're probably familiar with this process: if you put a drop of vegetable oil in water, the oil coalesces into a drop, separating from water (Fig. 2.3). Cells do the same thing, but at a smaller scale and with far more control. In cells, the lipids aggregate to form a lipid bilayer: a continuous sheet composed of two layers of aligned lipids (Fig. 2.9). All the tails pack side-by-side in the center and the heads are displayed on the two faces, comfortable in the surrounding water.

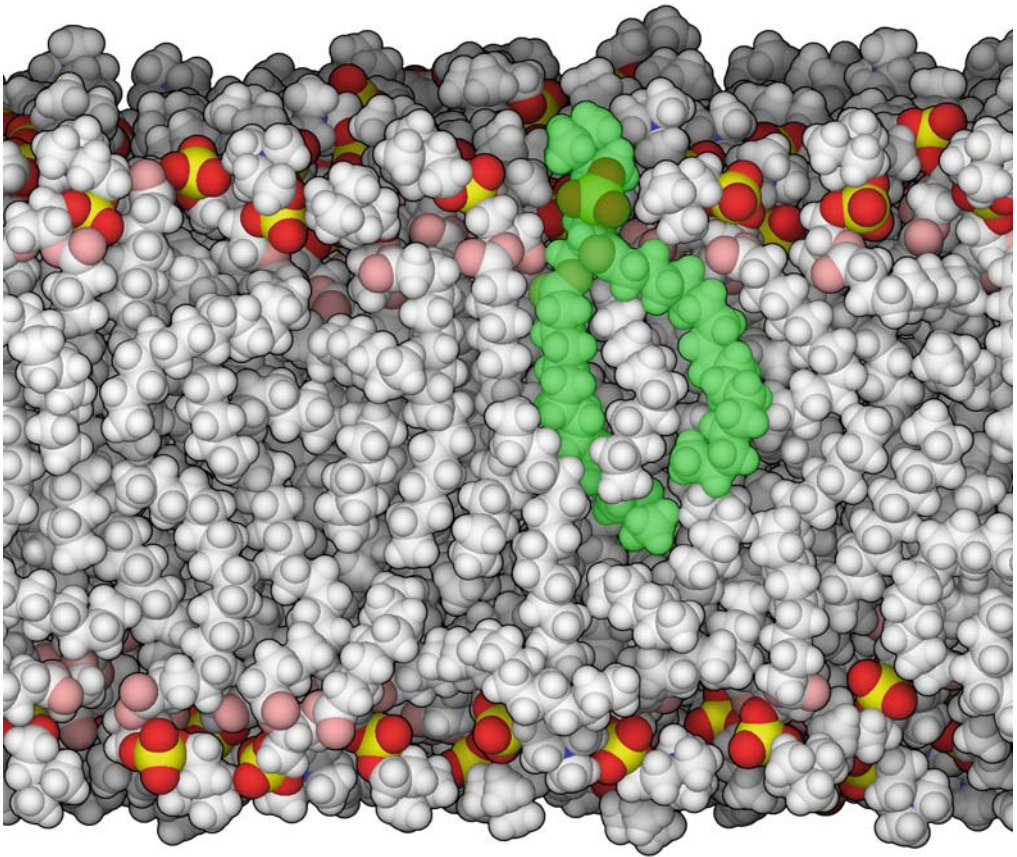


Fig. 2.9 Lipid Bilayers An individual lipid (highlighted in green) is small as compared to proteins and nucleic acids, and far more hydrophobic. In the cell, many lipids associate together to form a dynamic lipid bilayer. The bilayer is shown in cross-section here, revealing the tangle of hydrocarbon chains sheltered inside (20,000,000 X)

Since lipid bilayers are composed of so many separate molecules, they are dynamic and fluid. Each individual molecule rotates like a top, and its hydrophobic tails wag and flail. Lipids also slide rapidly past one another, always staying in the sheet, but randomly migrating sideways. Since they are so fluid, lipid bilayers make the perfect skin for cells. The membrane is flexible and bends easily to meet the demands of the cell. Rips in the membrane rapidly heal and the membrane can quickly grow or shrink in size simply by adding or removing more lipid molecules.

Lipid bilayers are also excellent barriers, because the lipid tails interact tightly inside. All of the cell's large molecules, like proteins and nucleic acids, are completely blocked and may be corralled safely inside the cell by a lipid membrane. Metal ions also cannot pass through the membrane and water molecules cross only rarely. However, small hydrophobic compounds, such as alcohol and drugs, pass right through, squeezing past the tails and easily gaining entry into the cell.

Pure lipid bilayers, however, are rarely found in modern cells. After all, a perfect barrier would seal the cell away from food and nutrients, and seal in waste materials. To solve this problem, cells build a variety of specialized proteins that are inserted into the membrane. They act as pumps that transport materials across the sealed membrane, or messengers that form a line of communication from one side to the other.

Polysaccharides

The last of the four basic building materials is used mainly for its texture. Polysaccharides are long, often branched, chains of sugar molecules. Sugars are covered with hydroxyl groups (an oxygen atom and a hydrogen atom) that interact nicely with water and also interact strongly with other hydroxyl groups. Polysaccharides take advantage of this property to perform two key functions. The first is storage. Sugar, glucose in particular, is the major source of energy used in the cell. Polysaccharides are used as the central storehouse of this energy. In lucrative times, surplus sugar molecules are linked up into large granules of polysaccharide. In tougher times, these granules are broken down and the sugar is released. Polysaccharides are less reactive than individual sugars, and the many hydroxyl groups associate to form compact, easily stored granules, making them better for storage of energy than high concentrations of free sugar. In plants, glucose is

stored in the form of starch, the same starch we used to thicken sauces and stiffen collars. In our own cells, glucose is linked in a slightly different way to form glycogen.

Polysaccharides play a second important role in cells. They are used to build some of the most durable biological structures, as well as some very gooey ones. The building where you are sitting and the pages of this book are made largely of polysaccharide. The cellulose fibers in wood are largely composed of long polysaccharide chains (Fig. 2.10).

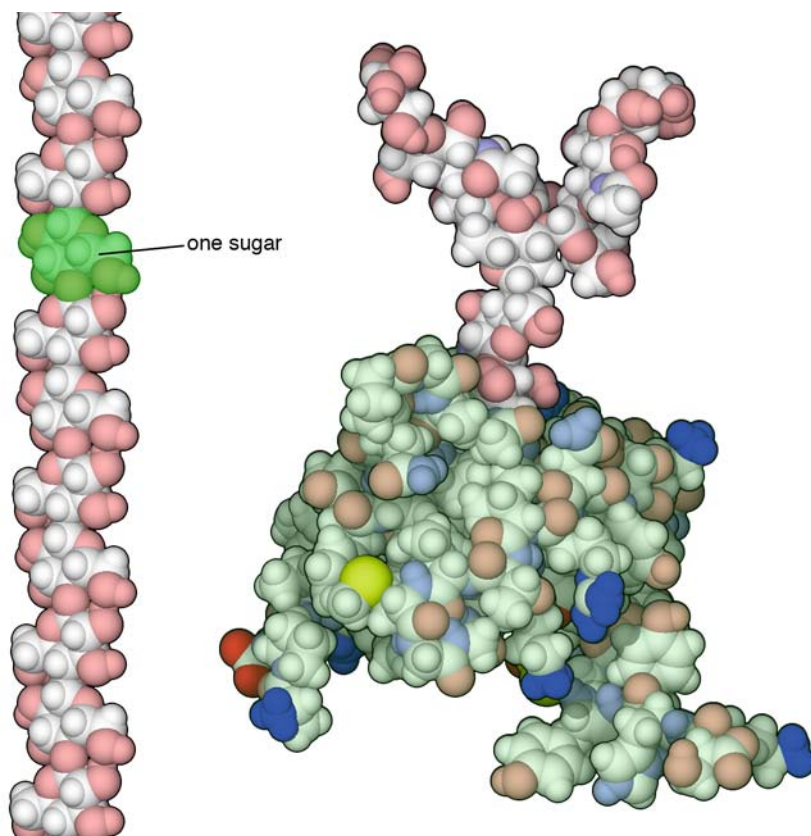


Fig. 2.10 Polysaccharides Polysaccharides are covered with hydroxyl groups (colored pink here), making them hydrophilic and soluble in water. Two examples are shown here. Cellulose is shown on the left, with one sugar highlighted. A protein hormone (chorionic gonadotropin) is shown on the right. It is composed of a short protein chain, tinted green here, and a branched polysaccharide chain. Small polysaccharides like this are attached to many of the proteins on the surface of our cells (20,000,000 X)

The stiff shells of insects are also made of long polysaccharides called chitin. These chains form tough girders, all glued together by the many hydroxyl groups, which give trees and lobsters their strength.

Our cells also use polysaccharides as a structural material, but much smaller ones than cellulose or chitin. Most cells are coated with a layer of short polysaccharides, connected to proteins or lipids on the cell surface. These chains extend away from the cell and interact with a lot of water. This mixture of polysaccharide and water forms a gluey coat around the cell that acts as a protective barrier. To get an idea of what this polysaccharide barrier is like, you only have to think of the last time you had a cold: mucous owes its distinctive properties to polysaccharide chains attached to its component proteins.

The Strange World of Molecules in Cells

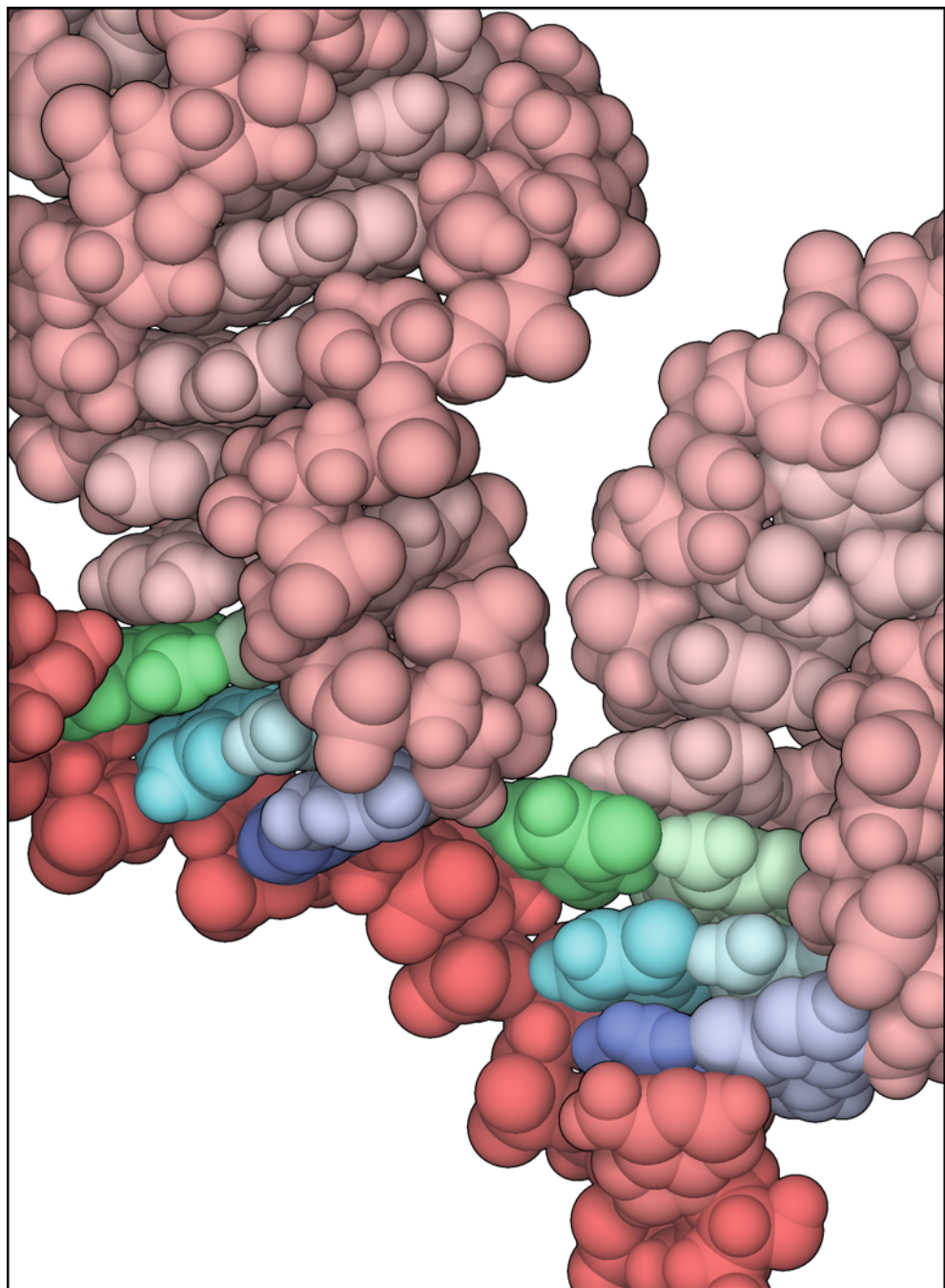
As we will see in later chapters, cells are small, crowded places, with many things happening at once. Molecular machines must perform their nanoscale tasks in this unusual environment, packed into living cells. This causes a number of surprising problems, and a few novel opportunities.

Cells are amazingly crowded, typically with 25–35% of the space filled by large molecules such as proteins and nucleic acids. As you might imagine, these molecules get in the way of each other. This has two seemingly opposite effects on the function of molecules. First, larger molecules have more trouble diffusing through the cellular environment, since they are constantly blocked by neighbors. This slows the motion of each molecule, so it takes longer for two molecules to find each other. However, countering this effect, crowded environments tend to favor the association of molecules once they have found each other. Since they are constantly crowded together by neighboring molecules, they spend more time next to each other and are far more likely to find the proper orientation for interaction. This property tends to favor association of molecules into big complexes in crowded environments, rather than filling the cell with lots of separate molecules.

Cells are also filled with barriers that both inhibit and enhance the motion of molecules. Membranes are often used to isolate a section of the cell, completely blocking the diffusion of molecules from one side to the other. This enhances the function of the enclosed molecule by sequestering it in a small space along with all of the other molecules

needed for its particular task. Membranes also speed up the diffusion of molecules and association of some molecules. If a protein binds weakly to a membrane, it can bind at one spot and then hop around on the membrane surface. Since this protein only needs to move in two dimensions on the surface, the molecule diffuses over a smaller region of space than if it were free to diffuse in three dimensions. So, it can find its targets on the membrane surface very quickly. This is also the case for proteins that bind to DNA—they can move rapidly up and down the helix finding the proper place. For instance, the lac repressor protein shows a weak, non-specific binding for most sections of DNA, and a very strong binding for the particular sequence of nucleotides it is designed to repress. Inside cells, by sliding up and down the helix, it finds its preferred binding site hundreds of times faster than expected with random, three-dimensional diffusion.

Molecular machines must also be very specific for the jobs that they do. Inside a cell, a typical enzyme will be battered by thousands of different types of molecules. It must be able to sift through these many distractions and grab the one that it needs. Biological molecules perform this daunting task by being experts at molecular recognition. They have very intimate interactions with their partners, making contact at many points and requiring a perfect match of shape and chemistry. Enzymes often completely surround their target molecules, and proteins associate through large complementary interfaces. Because of this remarkable specificity, a thousand reactions can be happening at the same time in a cell, and the whole thing can still be orchestrated by random association in the teeming cytoplasmic soup.



Chapter 3

The Processes of Living

What is life? We all know a living thing when we see it, but when you get right down to it, it's hard to come up with a consistent definition. For instance, plants grow and are alive, but crystals, which also grow, are not alive. What is the difference? In 1944, the physicist Erwin Schrödinger presented a very simple definition of life that has withstood the test of time. He identified one property that all life shares: living things avoid decay into equilibrium.

In our universe, there is a constant movement towards uniformity. A glass of hot water will cool until it is the same temperature as the surrounding air. That same glass of water will slowly evaporate and the water molecules will spread evenly through the room and out into the surrounding air. These water molecules may then join with many others and fall as rain, spreading the molecules over a wider and wider expanse. Even rocks, mountains, and entire planets are subject to this unstoppable force of decay and dilution—over millennia they weather and crumble, and ultimately they will be scattered as dust.

Living things resist these forces of decay. They strive to stay the same in spite of whatever challenges they face. Our bodies create heat when it is cold outside, resisting the natural tendency to cool down.



Fig. 3.1 Information Transfer The binding of transfer RNA to messenger RNA is the step where genetic information is translated into a sequence of amino acids in a protein. Here, two transfer RNA molecules (in light pink at the top) are bound to a messenger RNA (in darker pink at the bottom), reading two successive codons each composed of three nucleotides. The three bases in each codon/anticodon pair are colored green, turquoise, and blue. This process actually occurs while buried deep inside a ribosome, as shown in Fig. 3.4 (40,000,000 X)

Our skin guards our many component molecules, protecting them from the wind and the rain. Our cells constantly repair worn components or replace them with brand new ones, countering damage caused by environmental forces. The result is that, at least for the decades that we live, we remain more-or-less the same.

In this chapter, we will explore the basic methods that all living things on Earth use to postpone the inevitable decay into equilibrium. I have chosen to divide these into three basic categories. First, living things make all of their own component parts from things they can find in the environment, allowing them to grow, repair, and ultimately reproduce (Fig. 3.1). Second, living things use environmental sources of energy to fuel their continual battle against entropy. Third, living things insulate themselves from the environment, weathering hard times or moving to more fertile grounds. Solutions to these three problems were worked out in the early evolution of life, and today we rely on them to stay alive.

Building Molecules

Living cells require continual maintenance and repair. While carrying out the tasks of living—finding and digesting food, fighting with competitors, fleeing from predators—cells deftly replace any molecular components that become damaged or obsolete. Think about this feat for a moment—it is remarkable. You can't take your cells to a shop for repairs, like you would with a broken clock. Cells must make their repairs in place, without ever disturbing the ongoing processes of living. Imagine replacing a worn fan belt on your car, but doing it while driving down the road.

To make things even more challenging, cells must also be able to make all of their component molecular machines using only the resources that are available in the local environment. Think of the magnitude of this accomplishment. Many bacteria are able to build all of their own molecules from the a few simple raw materials like carbon dioxide, oxygen, and ammonia. A single bacterial cell knows how to build several thousand types of proteins, including motors, girders, toxins, catalysts, and construction machinery. This cell also builds hundreds of RNA molecules with different orderings of nucleotides, as well as a diverse collection of lipids, sugar polymers, and a bewildering collection of exotic small molecules. All of these different molecules must be created from scratch, using only the molecules that the cell eats, drinks, and breathes.

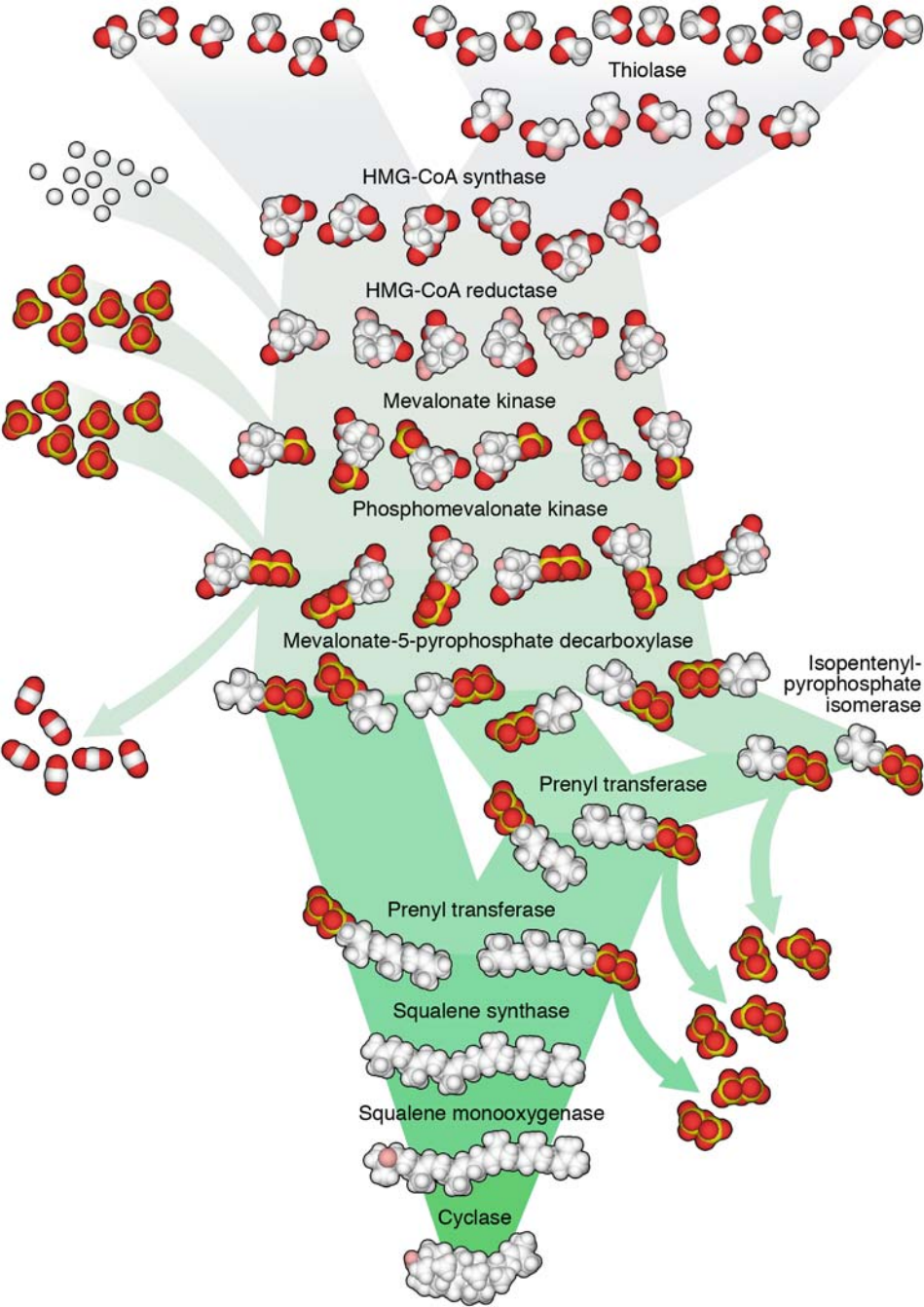
Our own cells are not quite that self-sufficient. We rarely need to go to those lengths, since we obtain many useful molecules in our diet. We cannot use energy from the sun, so we must eat sugars and fats for energy. To make proteins, we require a few amino acids in our diet that we cannot make from scratch ourselves. We also require vitamins—small molecules that we cannot make, but that are absolutely required by some of our enzymes (vitamins are discussed in more detail in the last chapter). We do, however, make hundreds of useful molecules, including lipids, most amino acids, nucleotides, and sugars. Our cells also make many unusual molecules for specialized tasks, such as thyroid hormones that include atoms, iodine, tiny nerve signal transmitters, natural painkillers, and bright red molecules that use iron ions to capture oxygen.

Living cells use two basic methods to create their component parts. All of the small molecules, like sugars and nucleotides, are constructed atom-by-atom through a series of chemical reactions. In many cases, it takes dozens of steps to convert the available raw materials into the final product (Fig. 3.2). The cell uses a collection of dedicated enzymes to perform these chemical reactions. These synthetic enzymes are fast, efficient, and specific; and they are highly regulated, so cells build only the molecules they need at the time they need them, without waste or side products. Because of these amazing enzymes, cells are masters of chemical synthesis, far exceeding anything that we can do with traditional chemical techniques in the laboratory.

However, this hard-wired approach is far too cumbersome for building thousands of different proteins, each of which must be constructed with a unique ordering of hundreds of amino acids. There is no way that a cell could contain a dedicated enzyme to build each individual protein. Instead, cells use a second synthetic method to build their proteins and nucleic acids—an information-directed method that is highly flexible and can be used to make any protein or any nucleic acid on demand.

The information-directed synthesis of proteins and RNA is the central molecular process that makes the diversity and tenacity of modern life possible. This method has two requirements. First, the final products must be constructed from a few standard components. In living cells, the components are amino acids or nucleotides, and the final products are proteins or nucleic acids. Second, a blueprint must be created that describes the order of these components in the final product. In every living cell on the Earth, the blueprint is stored in a sequence of nucleotides in the DNA genome.

In modern cells, this occurs in two steps. In the first step, called transcription, RNA molecules are made according to the information



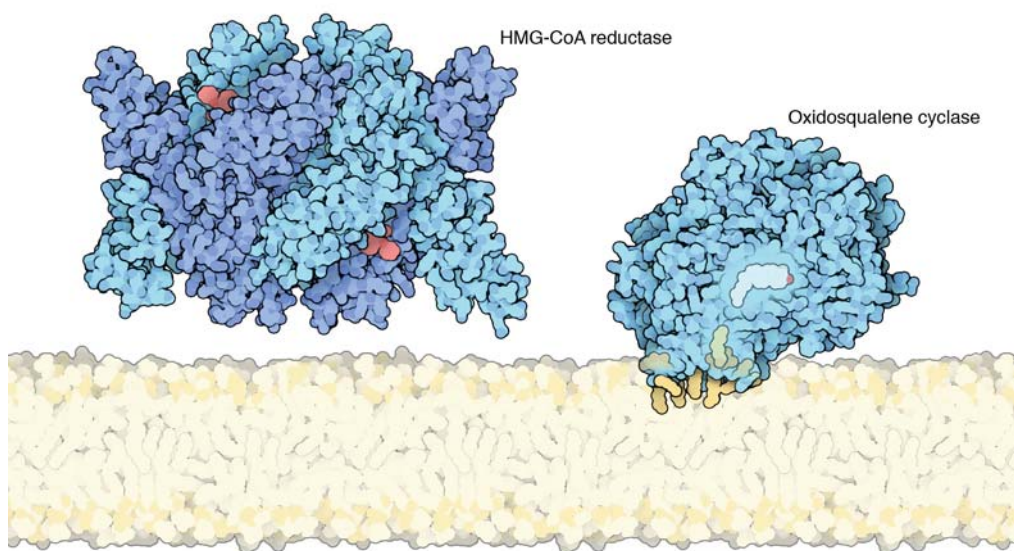


Fig. 3.2 Synthesis of Sterols Our cells build a lanosterol molecule from 18 molecules of acetate. As shown on the opposite page, a dozen chemical reactions are needed for this synthesis, each one performed by a dedicated enzyme. Lanosterol is then used to build cholesterol (requiring upwards of 20 more chemical reactions) and other steroid molecules. (10,000,000 X) Two of the enzymes used for sterol synthesis are shown above. HMG-CoA reductase performs one of the first condensation steps, setting the pace for the entire process. Oxidosqualene cyclase performs an amazing reaction that takes a long, thin oxidosqualene molecule and performs a cascade of reactions that fuse it into the chunky lanosterol. Oxidosqualene is very hydrophobic and is usually found inside a membrane, so the enzyme binds on the surface of the membrane and pulls the molecule into its active site (5,000,000 X)

stored in DNA (Fig. 3.3). The enzyme RNA polymerase unrolls a section of the DNA double helix and, at a rate of about 30 nucleotides per second, builds a strand of RNA complementary to it. The strand of RNA is a nucleotide-by-nucleotide copy of the DNA, containing exactly the same information as the segment of DNA. In cases where the RNA is the desired product, the process stops here and the RNA is released to perform its function.

Otherwise, the RNA copy is then used to direct the construction of the protein, in a process termed translation (Fig. 3.4). The sequence of nucleotides in the RNA strand is read and used to link amino acids in the proper order to form a new protein. Translation is more

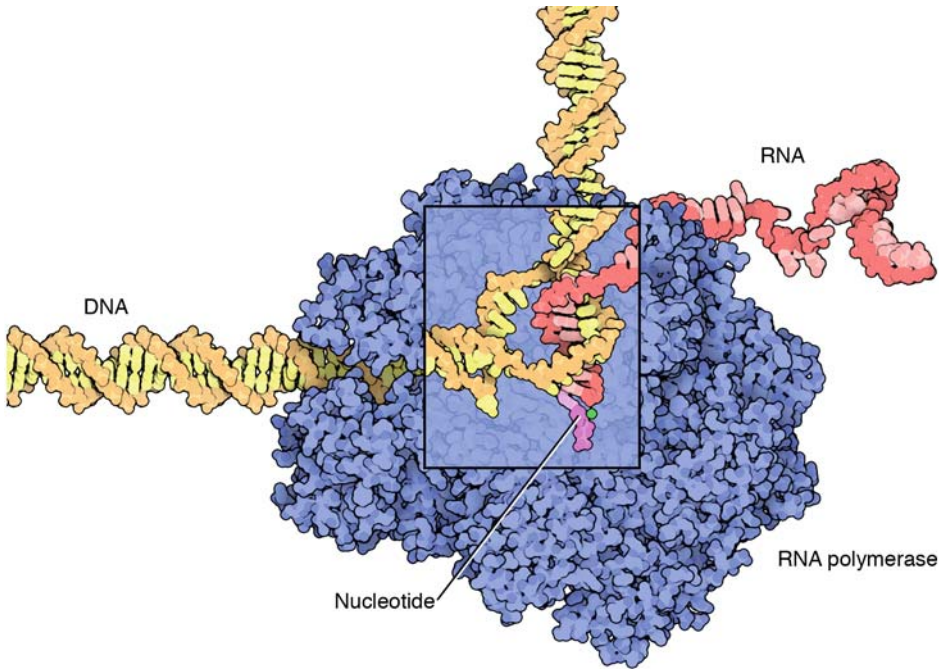


Fig. 3.3 Transcription of DNA to Build RNA RNA polymerase, shown in *blue*, unwinds the double helix of DNA and then builds a strand of RNA that matches the one of the DNA strands (5,000,000 X)

complicated than transcription, because there isn't a one-to-one correspondence of the nucleotides in RNA and amino acids in the new protein. There are only 4 types of nucleotides, and 20 different amino acids. Cells resolve this problem with the most conservative possible encoding: a triplet of nucleotides in the RNA, termed a codon, is used to specify each amino acid. For instance, the triplet C-U-G translates as leucine, C-G-G as arginine, and U-A-A is one of the three triplets used to encode "STOP!" The molecular details of this encoding make translation far more complicated than transcription, requiring the concerted effort of over 50 different molecular machines: some made of protein, some made of RNA, and some made of a combination of both.

The actual physical matching of each nucleotide triplet with its proper amino acid is performed by a special type of RNA, termed transfer RNA. Twenty different transfer RNA molecules are made with the different triplet of nucleotides at one end (termed the

anticodon) and the corresponding amino acid attached at the other end. A separate set of 20 different enzymes (amino-acyl tRNA synthetases) load the proper amino acid onto each type of transfer RNA. Ribosomes then bring everything together to build the protein. Chaperoned by special proteins that initiate and terminate the process, and other proteins that inject the energy for each step, ribosomes walk down a strand of RNA, align transfer RNA molecules alongside, and link up the amino acids they carry. At a rate of about 20 amino acids per second, an average protein takes about 20 seconds to build.

Information-directed RNA and protein synthesis is a remarkably nimble solution to molecular construction. All of the information is stored in the cell's genome; and when a new protein or RNA is needed, the information is read and used to direct its construction. But of greater importance, you can build entirely new proteins and RNA by simply modifying the instructions in the genome. You don't need to make an entirely new set of enzymes to construct a new protein—you just change the instructions and the protein synthesis machinery builds it for you. Comparison of genomes of different organisms has shown that all manner of mutations, cutting, splicing, and editing has been performed on this information over the evolution of life. Some essential proteins, which perform a central task, have remained largely unchanged for billions of years. For instance, the enzyme glyceraldehyde-3-phosphate dehydrogenase, shown in the first chapter, is virtually identical in human cells and in bacteria. Other proteins, however, are remodeled every single day. For instance, our antibody genes are shuffled and edited constantly to create new, fine-tuned antibodies to attack new infections.

The genome contains the recipes for building all of the cell's proteins, as well as many-layered instructions for how, when, and where to use this information. The entire genome sequence is known for dozens of organisms, including the *Escherichia coli* bacteria and human cells that are pictured in the following chapters. These genomes range widely in size and complexity. The smallest genomes appear to encode about 500 proteins—the genome of the parasitic bacterium *Mycoplasma genitalium* has 580,000 nucleotides that together encode 517 genes, and closer study of this organism has shown that about 300 of these are absolutely essential for life. The *Escherichia coli* genome is considerably larger, with about 4.7 million nucleotides and over 4500 genes, and our own genome has over 3 billion nucleotides and is estimated to encode about 30,000 proteins.

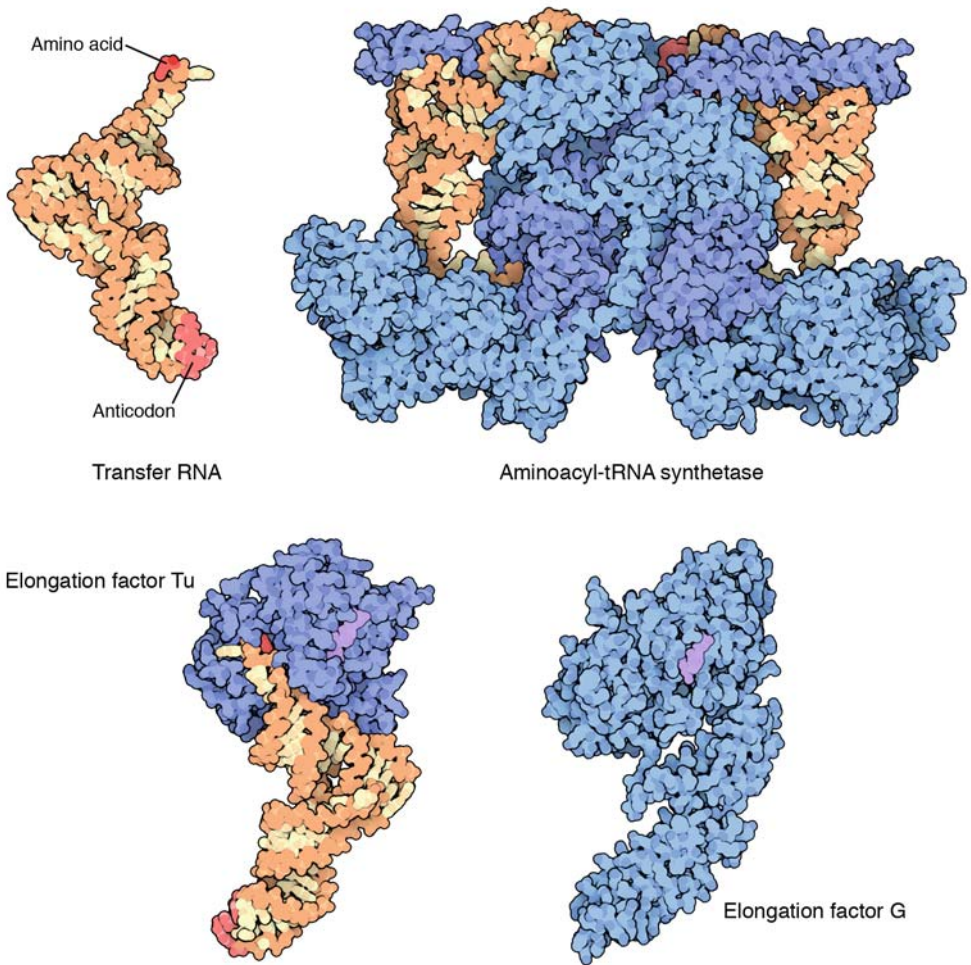
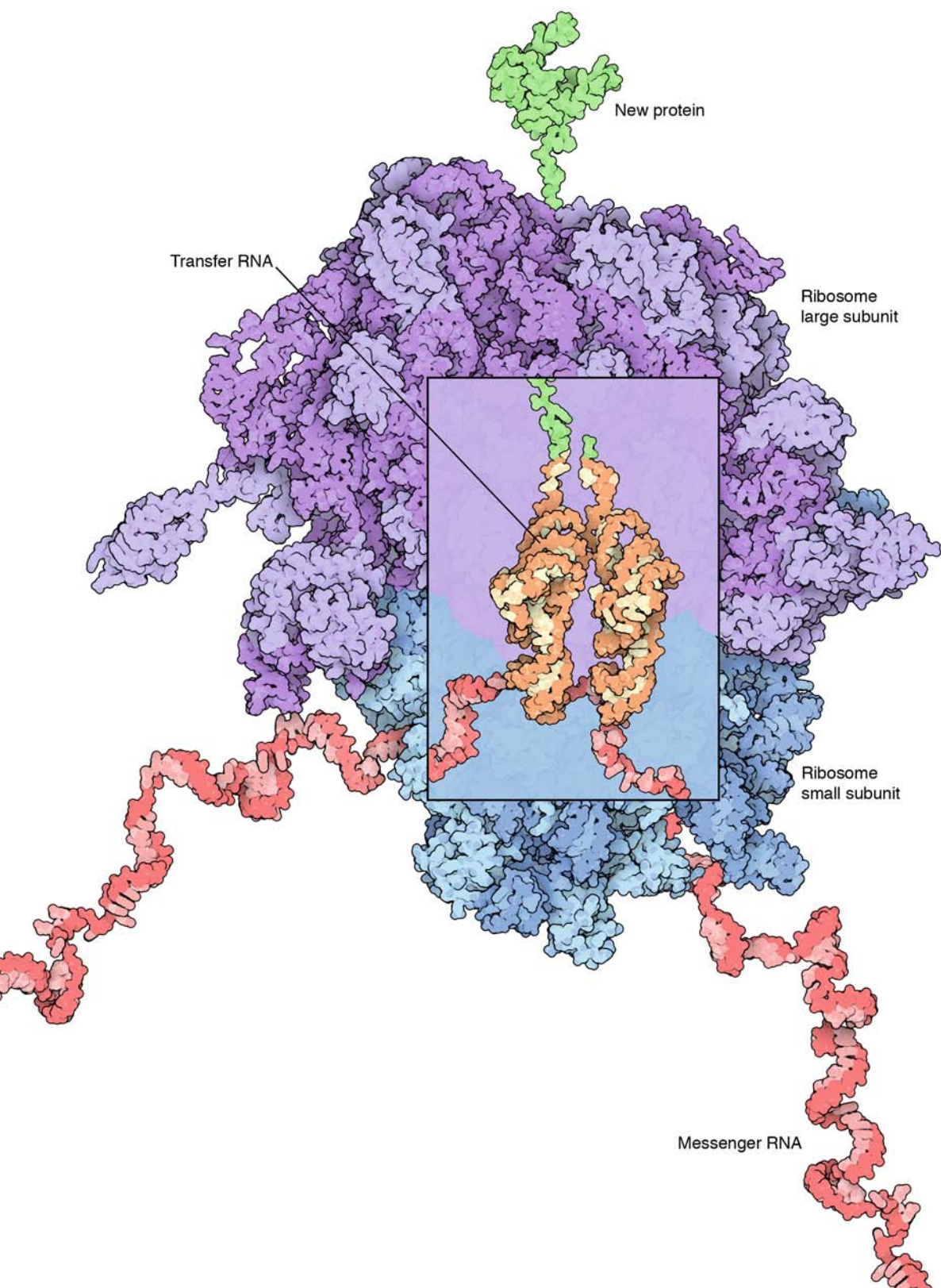


Fig. 3.4 Translation of RNA to Build Protein Protein synthesis requires the concerted effort of dozens of different proteins. The transfer RNA that carries a phenylalanine amino acid is shown above, along with phenylalanyl-tRNA synthetase, the enzyme that attaches the phenylalanine to end the transfer RNA. Nineteen other transfer RNA molecules, each with their own dedicated synthetase enzyme, are built for the other 19 amino acids. Many proteins assist with the process. In bacteria, elongation factor Tu delivers the transfer RNA to the ribosome and provides energy, and elongation factor G pushes the messenger RNA strand ahead by one codon as soon as each amino acid is added to the chain. The ribosome, shown on the opposite page, brings all these molecules together, aligning the proper transfer RNA molecules and connecting the amino acids together (5,000,000 X)



Bacterial genomes are relatively organized documents. The genes are arranged next to one another, often organized into function-specific operons with regulatory information in between. Our own genome, however, is very complex and disorderly. Each of the protein-encoding genes is interrupted by long segments of DNA, so that a typical gene is often 10 or 20 times longer than needed to encode the protein (Fig. 3.5). This is useful, however, when the gene is transcribed, the RNA copy must be edited to remove these segments, allowing many interesting variations and a whole extra level of control and regulation. Over half of our genome is also filled with segments that do not encode proteins, including huge tracts of repeated sequences and many mobile elements that jump from place to place.

Looking at the genomes of different organisms, we see that there are two opposing forces shaping the genetic information. On one hand, genomes must faithfully propagate the precious genetic information from generation to generation. On the other hand, mutation of the genome plays an essential role in evolution. It is the balance of these two that shapes the form and diversity of life, making sure that organisms breed true, but that there is enough diversity to allow adaptation to changing environmental needs. If we compare our genome with *Escherichia coli*, we find that they are widely different. A few highly conserved proteins, like the enzymes involved in the breakdown of sugar, are recognizable, but most things have changed substantially since our ancestors took their separate evolutionary paths. If we look at our closest relatives, however, our genomes look eerily similar. For instance, our own genome is only about 1% different from that of our nearest relative, the chimpanzee. However, if you start looking at the numbers of copies of each gene (which will change how they are expressed), the ways genes are inserted into genomes or deleted from them, and the mysterious functions of vast tracts of non-coding DNA, the differences are actually far greater. These differences have subtle but essential effects on development, shaping our bodies differently than the chimpanzee's.

The genome is not the only piece of information needed to understand a living cell, however. The genome is only part of the picture, and is only useful when examined in the context of a living cell. To build a comprehensive recipe for a living cell, we must also understand all of the proteins that are built from the genome—often termed the proteome. We must understand how these proteins are assembled and how they interact—termed the interactome. We must understand all of the other molecules in the cell—the self-assembled lipid membranes, the many different RNA molecules, the varied polysaccharides, and

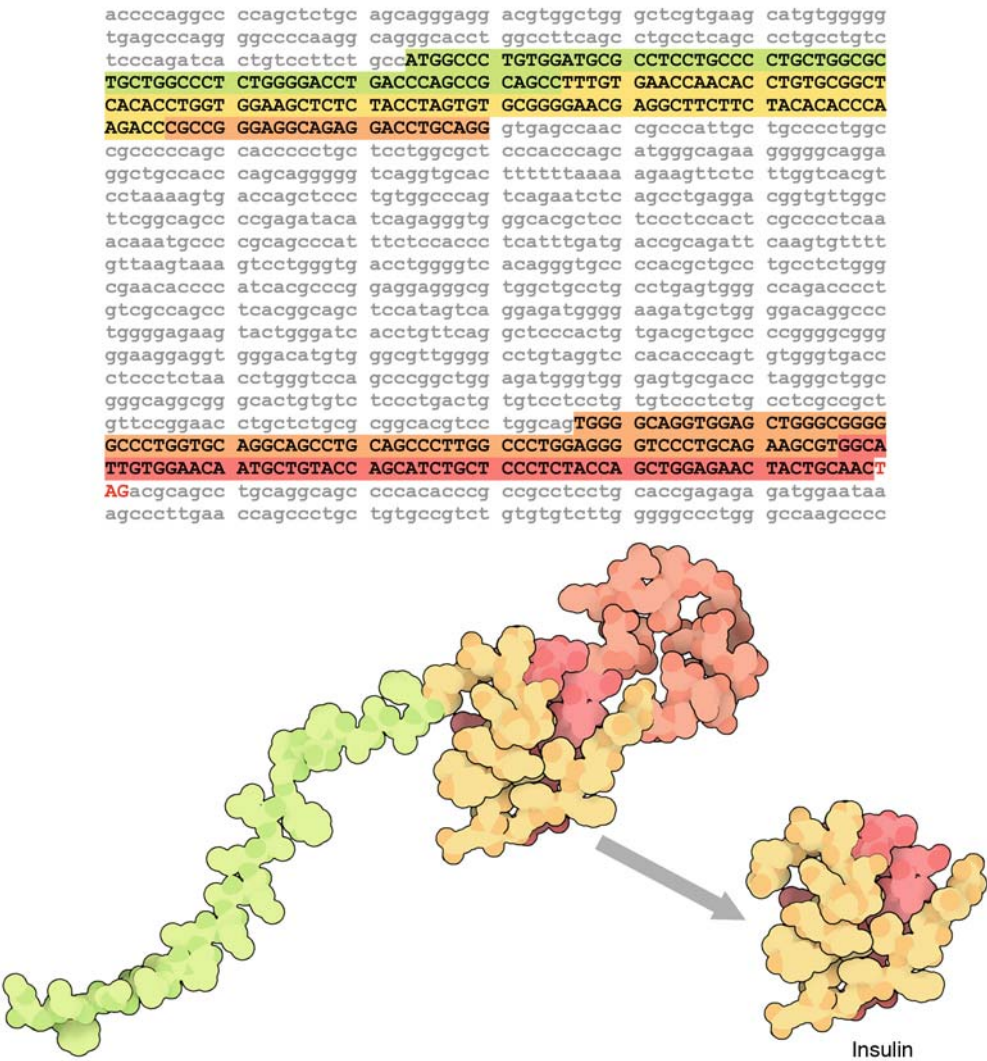


Fig. 3.5 Gene for Human Insulin A short segment from the human genome is shown here, including the gene that encodes insulin. Several layers of processing must occur to create the mature form of the protein. The protein-encoding sequence is broken into two segments in the DNA, shown here in capital letters. Notice that it starts with A-T-G (the typical start signal) and ends with the stop codon T-A-G. After the messenger RNA is transcribed and spliced to connect these two segments, a long protein with 98 amino acids is synthesized. This is then trimmed, removing the signal sequence at the start (shown in green) and a loop in the middle (shown in orange). The two remaining pieces form the mature protein (shown in yellow and red) (10,000,000 X)

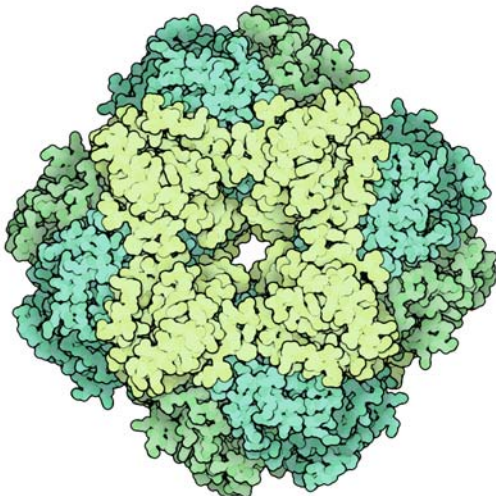
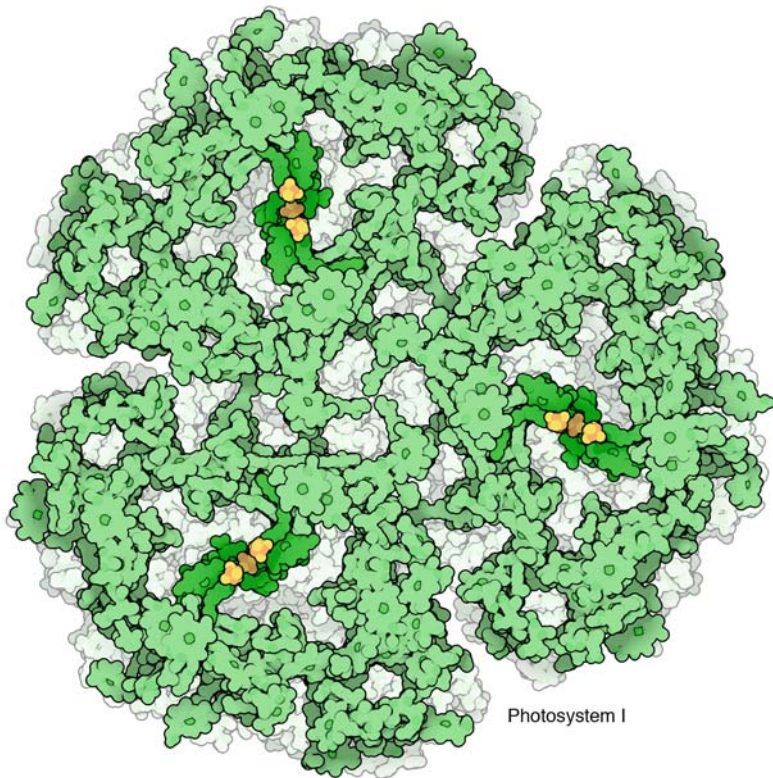
the hoards of small molecules racing between these larger molecules. Through careful scientific study, these many pieces of the puzzle are being fit together, providing a full recipe for building a living cell.

Harnessing Energy

Living things burn up a lot of energy in their constant battle to stay alive. A few uses of this energy are obvious: our bodies use a lot of energy when we move, and we need a lot of energy to keep our bodies warm. But there are other, smaller, processes that also require energy. We need energy to control the chemical reactions in our cells, ensuring that only the proper reactions occur at exactly the time they are needed. Tiny molecule-sized motors and pumps use energy to transport materials around the cell, delivering goods in a timely manner. A constant flow of energy allows cells, and entire organisms, to resist the natural tendency to cool down, come to rest, and slowly but inexorably erode.

The major source of energy on the Earth is the Sun. Some exotic bacteria power themselves with unusual reactions of hydrogen gas, sulfur, or ammonia, but most forms of life ultimately rely on sunlight to provide the energy for living. Plants capture sunlight and through the process of photosynthesis, they use the energy to combine carbon dioxide and water to create sugar molecules (Fig. 3.6). These sugar molecules are then used by the plant, and ultimately by most animals, bacteria, and fungi, to power the many processes of the cell.

Much of the energy in our cells is obtained through the breakdown of the simple sugar glucose. The carbon and hydrogen atoms in glucose molecules are broken apart and combined with oxygen to form water and carbon dioxide. This is why we need the oxygen in the air that we breathe—without this oxygen, we can't produce the energy needed to power our cells. The combination of glucose and oxygen is a powerfully energetic process. We are all familiar with this because a similar reaction is occurring when we burn wood, which is largely composed of long chains of sugars like glucose. However, our cells cannot simply burn food like a stove: all of the energy would be released as a burst of heat and the energy could not be captured and harnessed. Instead, cells take a less direct approach. Cells manage their energy in small portions, breaking down glucose molecules in



Ribulose biphosphate carboxylase/oxygenase

many successive steps, each under perfect control and each involving only small changes in energy.

Cells wield many types of energy, including chemical energy, electrochemical energy, physical motion, absorption and emission of light, and the flow of electrons. As with the breakdown of sugar, molecular machines perform these energetic processes in small steps. Chemical energy is obtained through reactions of single molecules. Electrochemical energy is stored by moving individual ions, light is captured one photon at a time, and electrons are moved one-by-one along a string of molecular electron carriers. This allows a level of control, and efficiency, that is rarely seen in our familiar macroscale world.

Chemical energy is obtained by performing particularly energetic chemical reactions, which are then harnessed to power other, less-favorable processes. ATP (adenosine triphosphate) is the primary currency of chemical energy (Fig. 3.7). It is used to capture chemical energy and transport it to places where it is needed. ATP is a nucleotide, like the ones used to build DNA, with a string of three phosphates attached at one end. Each of these phosphates carries a negative charge, so they strongly repel one another. This makes it difficult to construct ATP, but easy to break it apart. The cell takes advantage of this by building ATP when it has a rich source of energy, and breaking it when it needs to power a process that needs some energy. For instance, a few of the reactions involved in the breakdown of glucose are particularly favorable, so the enzymes that perform these steps force two reactions to happen at the same time: the favorable breakdown reaction is linked to the construction of ATP. In other cases, a



Fig. 3.6 Photosystem and Ribulose Bisphosphate Carboxylase These two proteins, along with a collection of other proteins to assist them, provide most organisms on the Earth with food. The photosystem absorbs light using brightly colored chlorophyll molecules, and then use the energy to power an energetic flow of electrons. In this illustration, only the chlorophylls and other cofactors are shown—in reality, they are surrounded by protein chains. The main light-powered reaction occurs at the center of each of the three subunits, using a special relay of chlorophyll molecules and iron–sulfur clusters (colored brightly here). The many surrounding chlorophyll molecules act as antennas that gather light and funnel the energy into the center. The energy is ultimately used, after a few conversions, by the enzyme ribulose biphosphate carboxylase/oxygenase to make digestible sugar molecules out of carbon dioxide (5,000,000 X)

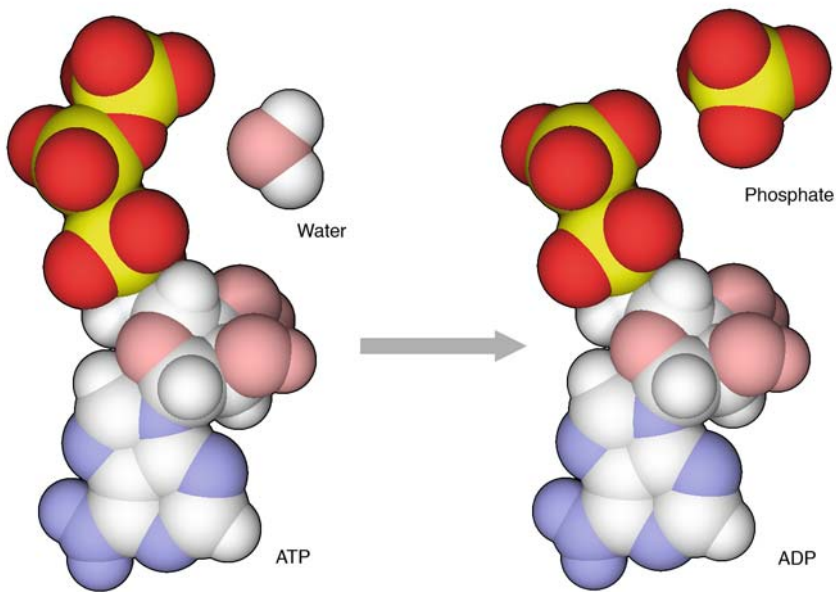


Fig. 3.7 ATP (Adenosine Triphosphate) ATP is unstable because the three phosphate groups, each of which carries a negative electrical charge, are bonded directly together. The reaction shown here, with water breaking the bond between two of the phosphates, is highly favorable and is used to power many processes in the cell (30,000,000 X)

sluggish reaction, such as the bonding of amino acids to transfer RNA (Fig. 3.8), is sped up by linking it with the breakage of ATP.

Cells also power themselves with cell-sized electrochemical batteries. Charged ions, such as hydrogen ions or sodium ions, are pumped across the membrane, building up on one side. This creates an electrochemical gradient, like a charged battery, that may be used to power other machines by allowing the ions to travel back across the membrane, evening out the levels. Many methods are used to pump these ions across membranes, including the breakage of ATP, absorption of light, or an energetic flow of electrons. These electrochemical gradients are used for many things, for instance, a gradient of sodium ions is used in nerve signaling and a gradient of hydrogen ions is used to power the production of most of our cell's ATP.

Physical energy is used in many specialized tasks. Proteins are dynamic machines, and different shapes can have entirely different

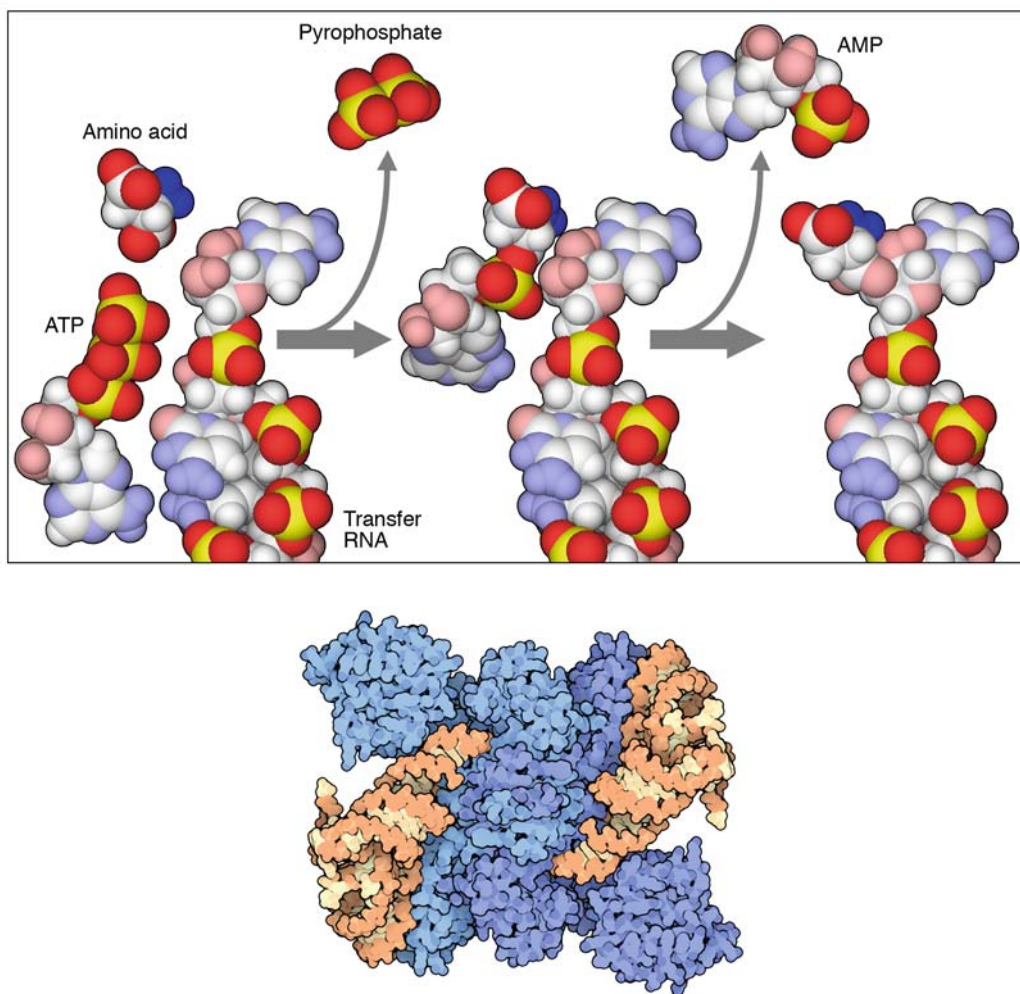


Fig. 3.8 Usage of ATP ATP is used to assist many reactions, such as the addition of amino acids to transfer RNA used for protein synthesis. The enzyme, aspartyl-tRNA synthetase, is shown at the bottom with two transfer RNA molecules bound to it. The tips of each transfer RNA bind deep into the active site of the enzyme, where an aspartate amino acid is added. The reaction occurs in two steps, as shown at the top, powered by cleavage of ATP. In the first step, two phosphates are broken from the ATP, and the remaining part binds to the amino acid, activating it. In the second step, the activated amino acid is attached to the transfer RNA, with the release of AMP (top, 20,000,000 X; bottom, 5,000,000 X)

functions. Some of this motion is used at a visible scale, like the motion of muscle proteins that moves our arms and legs. Most of the motions, however, are much smaller. For instance, repressor molecules use very small changes in shape to switch on and off—one shape fits perfectly to the DNA double helix and blocks it, but a slightly wider shape doesn't fit at all, and falls off allowing the gene to be read.

These different forms of energy are often interchanged for specialized tasks. For example, the motor myosin uses the cleavage of ATP to power the motion of muscles, converting chemical energy into physical motion. Bacteriorhodopsin absorbs light and uses the energy to pump hydrogen ions, creating an electrochemical gradient. The protein ATP synthase interconverts three forms of energy, transforming electrochemical energy into physical rotation and finally into chemical energy. The process of cyclic phosphorylation (Figs. 3.9 and 3.10), used by plants to create ATP powered by light, combines all of these types of energy.

Protection and Perception

When interacting with the world, we must isolate and protect our bodies from the surrounding environment, but at the same time, we need to sense and respond to changing conditions. Modern organisms have developed a bewildering variety of molecular machines to balance these two opposing needs. This is where the full diversity of life is expressed. The basic methods of molecular construction and energy production, discussed in the previous sections, are very similar in all living cells. But organisms have evolved many different and unique ways of dealing with the challenges of their diverse environments. The mechanisms used to insulate a cell from salty seawater are entirely different from the methods for protecting against desert heat or thin mountain air. Novel combinations of molecular machines allow an organism to swim after prey, to run away from a predator, or simply to taste really bad.

One common motif is used by all living cells: a lipid bilayer forms the primary boundary surrounding a cell and separating it from the surrounding environment. Lipid bilayers are flexible, self-healing barriers that are resistant to the passage of most molecules. The cell membrane keeps the cellular machinery inside and dangerous molecules out. However, a perfectly sealed skin would be useless to a cell.

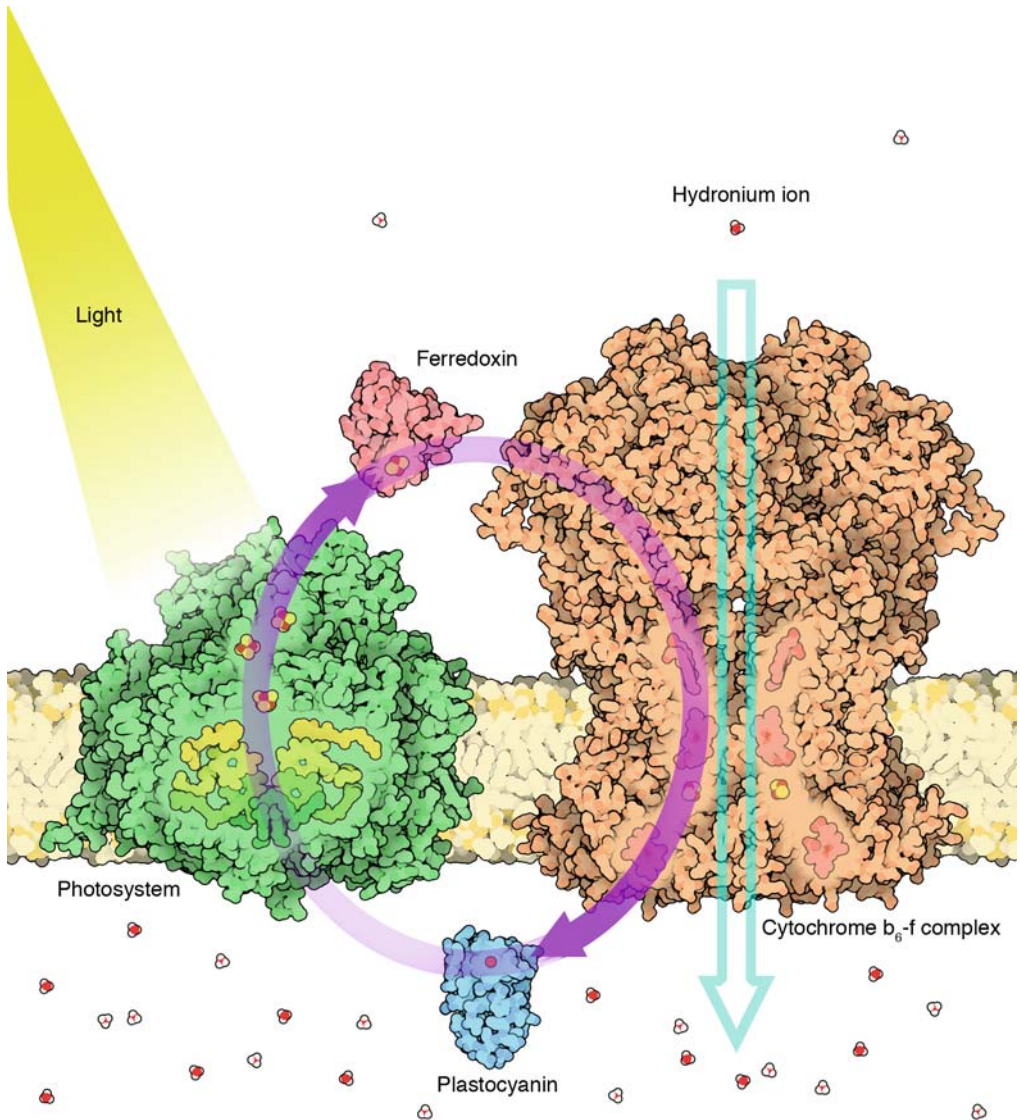


Fig. 3.9 Cyclic Photophosphorylation Plants use the energy of light to make ATP, but they must go through many energy transformations to do it. The process starts with a photosystem protein that captures individual photons of light, and uses the energy of these photons to create high-energy electrons. These electrons then flow through several proteins, hopping through a chain of iron ions and copper ions and slowly losing their energy until they return to the photosystem, ready for another charge from light. As the electrons flow through the cytochrome b_6-f complex, they are used to power the pumping of hydrogen ions across the membrane, creating an electro-chemical gradient. This gradient is finally used to power ATP synthase (5,000,000 X)

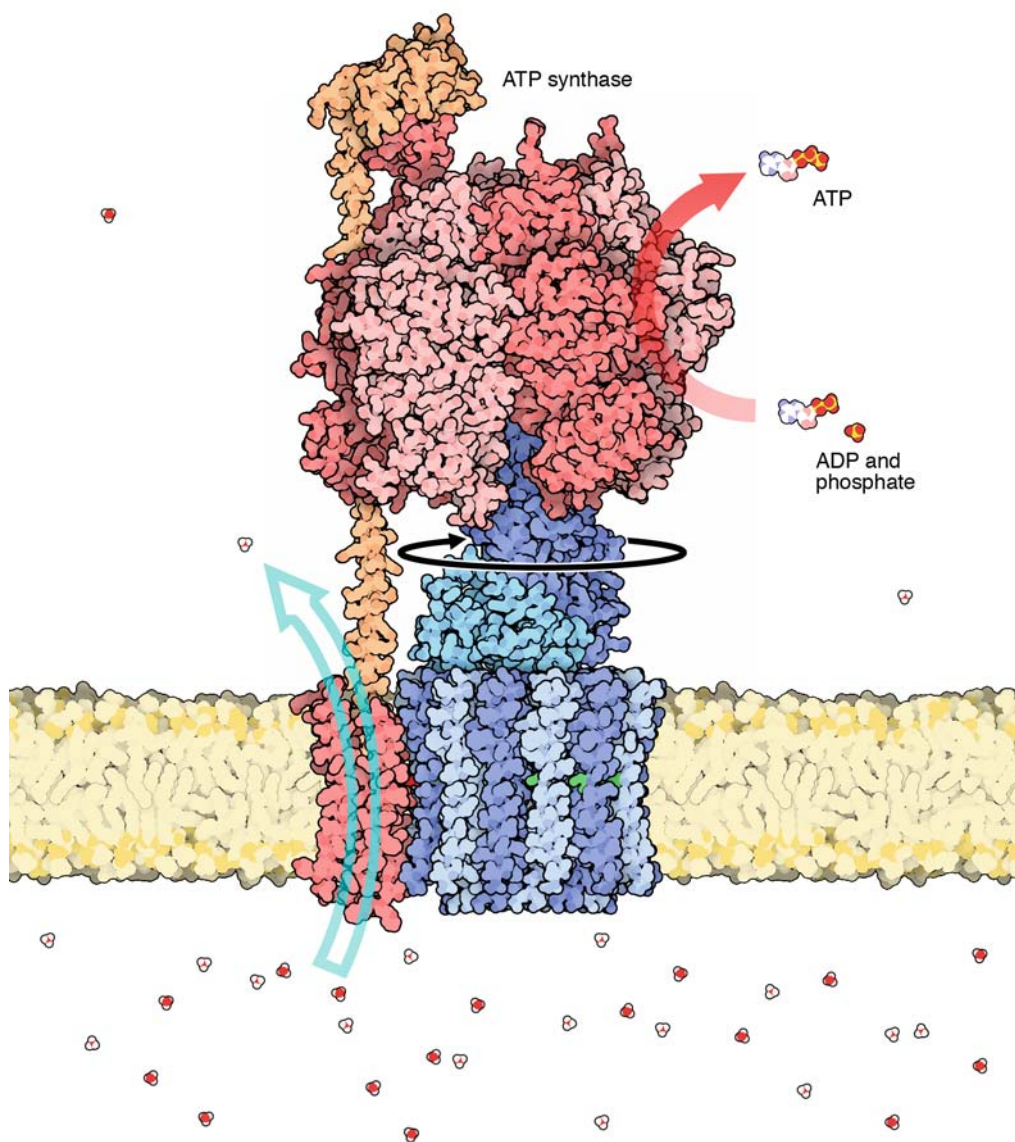


Fig. 3.10 ATP synthase is a molecule-sized generator that converts electrochemical energy into chemical energy. It is composed of two rotary motors connected together by an asymmetric axle. Flow of hydrogen ions through the bottom motor, which is embedded in a membrane, turns the large cylindrical rotor. This is connected to the axle, forcing it to turn inside the second motor at the top. With each turn, the axle distorts the subunits in the upper motor, catalyzing a reaction that makes the unstable phosphate-phosphate bond in ATP (5,000,000 X)

How would food ever get inside? Cells solve this problem by building protein pumps that span the membrane and carry molecules from one side to the other (Fig. 3.11). For instance, specialized pumps are built to pump amino acids in, others are made to pump the urea out, and others do nothing but trade sodium for potassium across the membrane. Using these pumps, cells carefully regulate the traffic of molecules across the membrane, transporting nutrients in and waste products out.

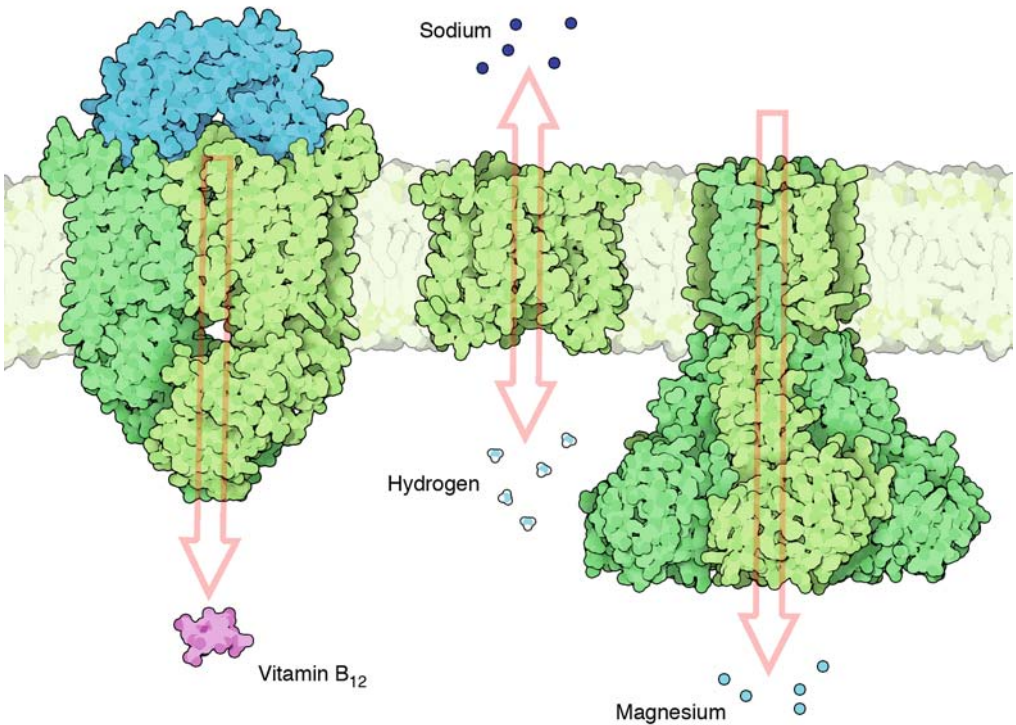


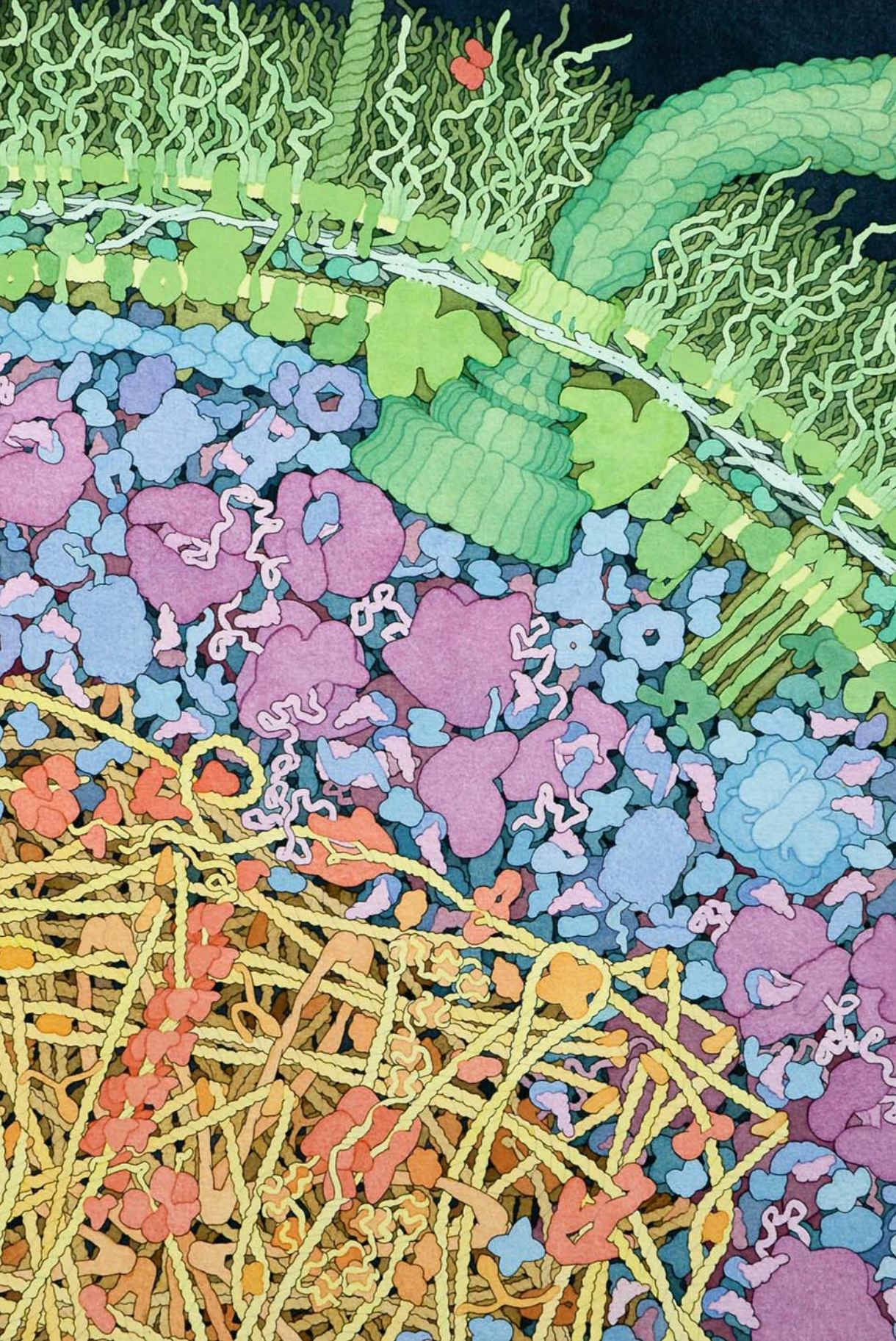
Fig. 3.11 Transport Across Cellular Membranes These three proteins are some of the many protein pumps found in the cellular membrane of *Escherichia coli* cells. On the left, vitamin B₁₂ is collected by the scavenger protein shown in blue, which delivers it to the pump for transport across the membrane. The protein at the center is an antiporter that transports hydrogen ions and sodium ions in opposite directions. The protein on the right delivers magnesium ions into the cell (5,000,000 X)

Lipid bilayers, however, are tenuous structures on a larger scale, so they must be buttressed to provide a strong protective wall against weather and predators. This is where we start to see some of the diversity developed through evolution to solve a problem. For instance, plant cells build a tough sheet of cellulose outside their lipid bilayers. This polysaccharide coat is so tough that it lasts long past the death of the plant, and can be used in wood and paper. The cells in our body, illustrated in Chapters 5 and 6, use an internal scaffolding made of protein, attached to points inside the lipid bilayer and linked to a mesh of protein fibers that run throughout the cell. The bacterium in the next chapter builds two concentric lipid bilayer membranes, with a tough sugar–protein sheath tethered in between.

Diversity is the rule when you look at the molecular machinery of perception and reaction. Of course, we would expect this since the environment is the single largest influence on the evolution of organisms. Through natural selection, each type of animal and plant has evolved to sense, exploit, and multiply in its particular environmental niche. For instance, we can contrast two distant relatives: the intestinal bacterium *Escherichia coli* and its host, ourselves. We span the spectrum of complexity in living organisms: the bacterium has minimal capability for perceiving and reacting to short-term changes in its environment, whereas the major portion of our body is devoted to these tasks.

Escherichia coli cells commit less than 5% of their molecular machinery to motion and perception, allowing only the simplest responses (described in more detail in the next chapter). Our bodies, in contrast, are built for specific, directed motion under the control of detailed, reasoned perception. The bulk of our body weight is dedicated to sense, reaction, and motion. Cells in our retina are filled with arrays of opsin proteins for sensing light, light that is focused by layers of eye lens cells packed full of clear crystallin proteins. Cells in our skin spin enormously long strands of keratin proteins into hairs, and other cells sense their slightest movement. These and other sensory data are transmitted and processed by nerve cells that carry electrical currents propagated by proteins and insulated by concentric layers of lipid. Fine control of movement is accomplished by an enormous skeleton of mineralized bone cells, moved by muscle cells filled with proteins that do nothing but contract, all glued together by connective tissue cells that build tough layers of sugar

and protein. However, the common thread of life on Earth still shows through this diversity, tying the simplicity of the bacterium to the complexity of our bodies. All of these unique molecular machines are built of the same four molecular components—proteins, nucleic acids, lipids, and polysaccharides.



Chapter 4

Molecules in Cells: *Escherichia coli*

When looking at cells, bacteria are a great place to start. Bacteria are small, sleek, and self-contained, they grow and multiply quickly when resources are plentiful, and they can mobilize powerful methods for weathering dry spells when the environment turns hostile. Some would say that bacteria are the most successful organisms on the Earth. They may be found in nearly any place, from freezing water to boiling hot springs, and they have found ways to exploit every possible source of sustenance.

When looking at bacteria, *Escherichia coli* is arguably the best choice for detailed exploration. *Escherichia coli* is currently the most-studied cellular organism known to science. It has been central to the study of biochemistry since its discovery in 1885 by Theodor Escherich, due in part to its general availability and ease of growth, and due in part due to plain serendipity. Study of *Escherichia coli* cells played a major role in many of the seminal discoveries of biochemistry, including the genetic code, glycolysis, and the regulation of protein synthesis. Through use of purified proteins and strains with mutant proteins, many of the basic processes of living have been studied in *Escherichia coli* cells.



Fig. 4.1 Inside a Bacterial Cell When we look closely at an *Escherichia coli* cell, we observe a bustle of organized activity. This illustration shows a portion of a cell magnified to where individual molecules may be seen. Only the large biomolecules are shown, including proteins, nucleic acids, polysaccharides, and lipid membranes. The spaces in between are filled with water, sugars, nucleotides, amino acids, metal ions and many other small molecules, as shown in Fig. 4.3 (1,000,000 X)

More recently, scientists have been looking at whole cells and trying to figure out the way that the parts work together. *Escherichia coli* was one of the first organisms to be fully sequenced, so we can now look at its genome to see the entire set of plans needed to specify all of its proteins. In addition, many analyses of the proteome and interactome have been performed, filling out our knowledge of the entire set of proteins made by the cell and the way these proteins interact and change under different environmental conditions. By combining this information with the many atomic structures of *Escherichia coli* biomolecules, we are very close to having a complete parts list and specification for this well-studied cell (Figs. 4.1, 4.2 and 4.3).

The most plentiful ingredient of a typical *Escherichia coli* cell is water, making up about 70% of the weight of the cell. The other 30% is composed of proteins, nucleic acids, ions, and all the other molecules. This might sound like a lot of water, but the cell is actually much more concentrated than most familiar liquids. For instance, egg whites are a gooey mixture of about 90% water and 10% protein—imagine the consistency of an even more concentrated solution. These tightly packed molecules come in all shapes and sizes. The genome encodes over 4300 different protein chains, and another 191 different RNA molecules. These perform an estimated 1250 enzymatic reactions and 255 transport tasks. About 1220 types of small molecules—including amino acids, nucleotides, sugars, and a host of others—circulate through the spaces in between the larger molecules. Obviously, this tiny cell is a busy place!

The Protective Barrier

A multi-layered cell wall surrounds each *Escherichia coli* cell (Fig. 4.4). It plays a variety of important roles, but foremost it is the protective barrier that insulates the machinery of the cell from environmental dangers. The outermost membrane is the cell's first line of defense. It is composed of a lipid bilayer with specialized lipids, called lipopolysaccharides, making up most of its outer face. Lipopolysaccharides are long strings of polysaccharide with a little bundle of lipids attached at one end. The lipid anchors the molecule into the membrane, and the polysaccharide chains extend into the surrounding liquid, forming a gluey, protective coat. Our immune system actually uses these lipopolysaccharides to recognize bacteria when they start to invade into the body. Antibodies recognize the lipopolysaccharides and mobilize our defenses to fight the infection.

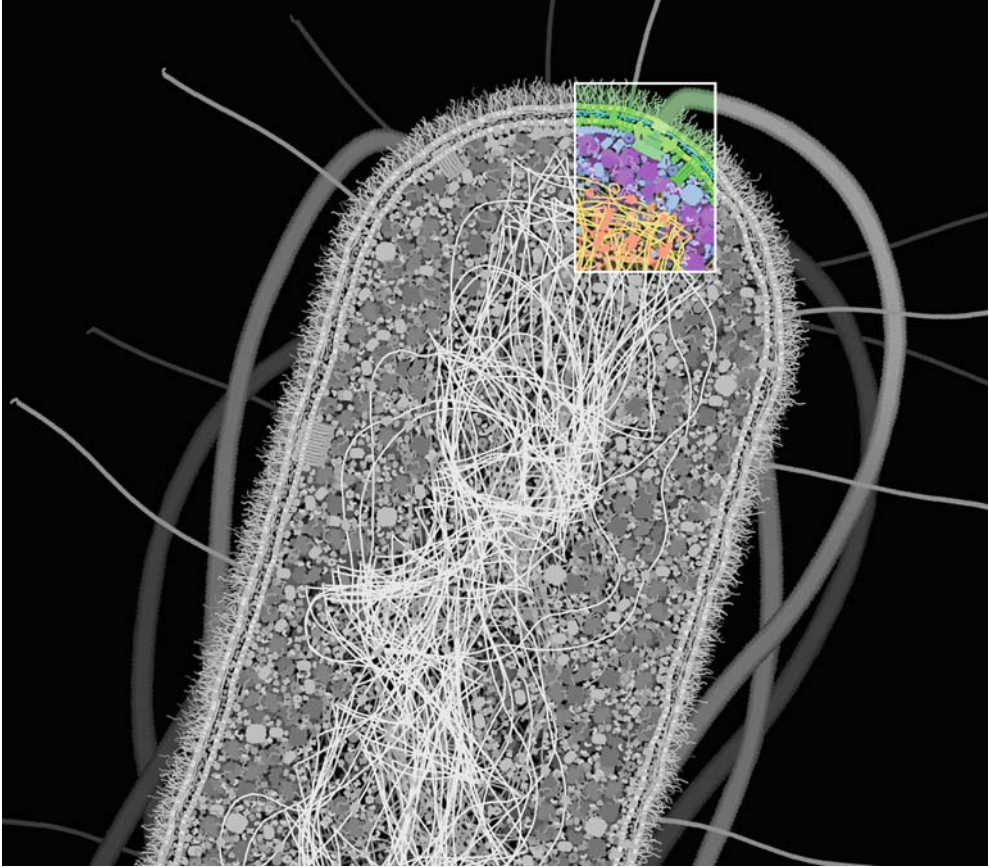


Fig. 4.2 *Escherichia coli* Cell The overall architecture of the cell is simple in concept but highly complex in details. Each cell is surrounded by a double-layered cell wall and all of the soluble components are packed inside. Long corkscrew-shaped flagella are turned by flagellar motors embedded in the membrane, and fimbriae extend at all angles, providing anchors if the cell finds a place to rest. The interior is separated roughly into two areas—a soluble portion that contains most of the ribosomes and enzymes and a nucleoid that is primarily filled with the tangled DNA genome. The boxed area is enlarged in the previous illustration. The whole cell is also shown in Fig. 1.1 (10,000 X)

The outer membrane, however, is not totally impervious. It is a coarse filter that blocks large dangers, but smaller molecules, like food molecules, pass easily through. The outer membrane is filled with porin proteins, which form small holes through the membrane. These holes are big enough for nutrients and ions to pass through, but small enough to keep the machinery of the cell inside. The outer

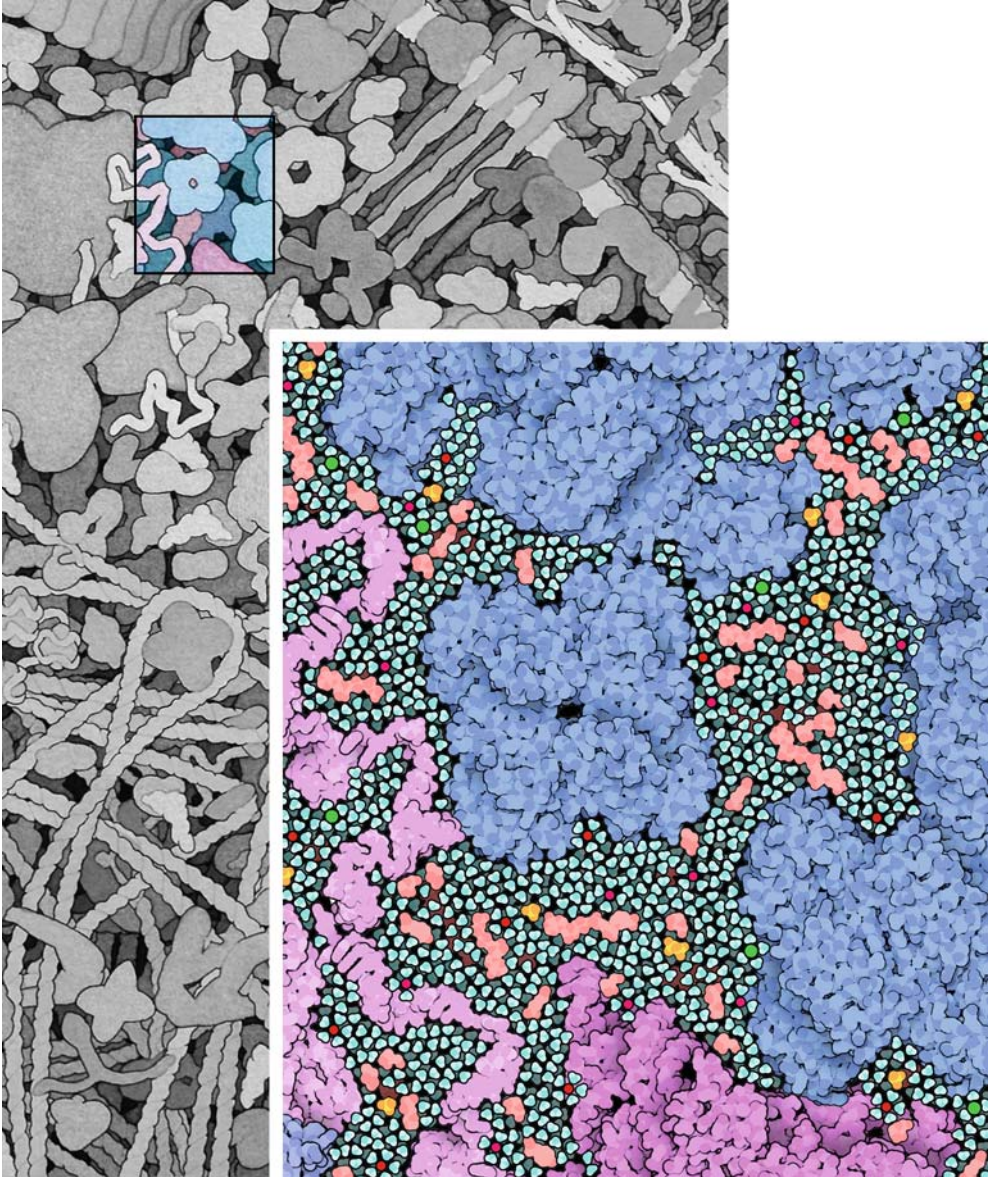


Fig. 4.3 Water and Small Molecules This illustration shows a small portion of the cell at a higher magnification, showing the crowding of small molecules between the larger proteins and nucleic acids. Amino acids, sugars, ATP, and many other small organic molecules are shown in pink. Metal ions are shown in bright red, phosphate ions are in yellow and orange, and chloride ions are in green. All of the remaining space is filled with water molecules, colored turquoise (5,000,000 X)

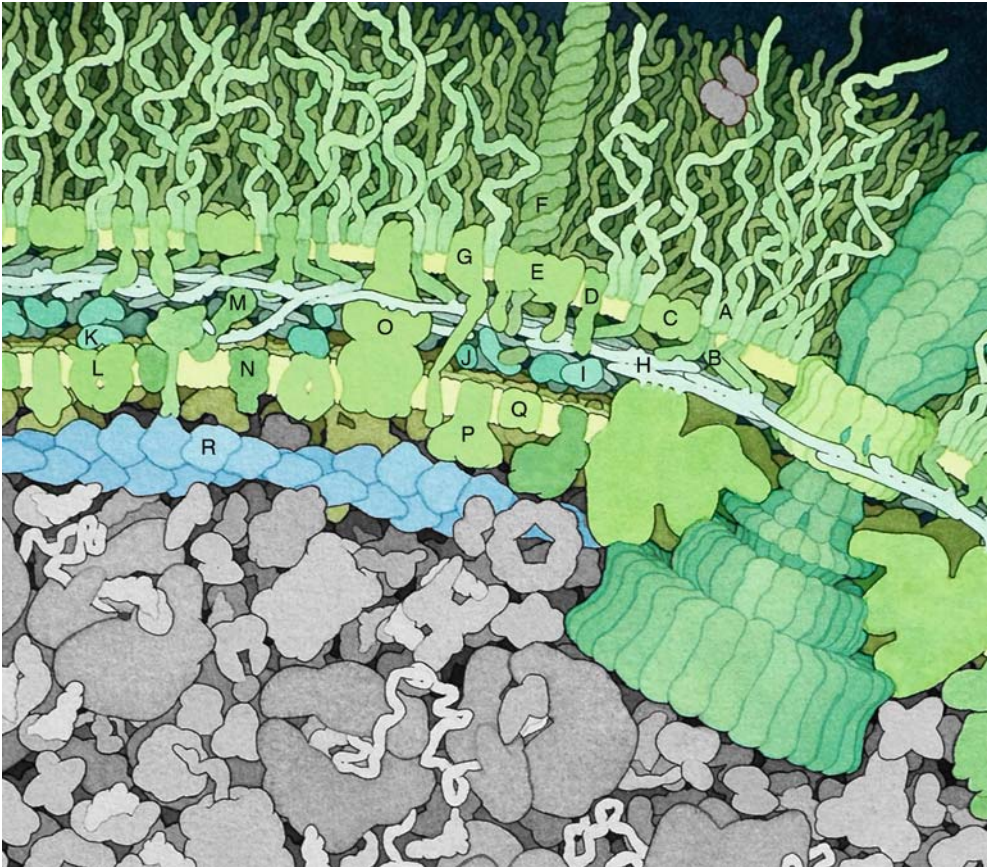


Fig. 4.4 The Cell Wall The cell wall is composed of two concentric membranes separated by a periplasmic space. Molecules in the outer membrane include lipopolysaccharides (A), lipoproteins (B), porin (C), OmpA (D), fimbrial ushers (E), fimbria (F), and the iron transport protein FhnA (G). The periplasmic space is filled with a supportive layer of peptidoglycan (H) as well as many small protective enzymes such as beta-lactamase (I) and superoxide dismutase (J). There is also a collection of periplasmic binding proteins (K) that deliver nutrients to transporters in the inner membrane, such as the vitamin B12 transporter (L). The inner membrane contains a bewildering variety of molecular machines, including the enzymes that build the peptidoglycan sheath (M), mechanosensory channels (N), the multidrug resistance transporter (O), ion transporters such as the magnesium transporter (P), and the sodium/hydrogen antiporter (Q). Surprisingly, bacterial cell walls are often supported by a simple cytoskeleton, such as the actin-like protein MreB (R)

membrane also anchors many fimbriae. These long, thin protein complexes are extruded piece-by-piece through special gateway proteins in the outer membrane. Fimbriae have sticky ends, and when the bacterium finds an amenable place to rest, they glue it in place. The type of fimbriae made by a particular strain is important. The fimbriae of pathogenic bacteria allow them to attach to human cells and resist attack by the cells of immune system.

The space between the two membranes, termed the periplasm, contains the major structural support that gives the cell its elongated shape. A tough layer of peptidoglycan, a cross-linked meshwork of polysaccharide and protein, is built just inside the outer membrane. This net-like bag envelops the whole cell, providing strength and support. The peptidoglycan sheath is anchored to the outer membrane by a several types of proteins, including small lipoproteins, which are composed of a small protein connected to a lipid, and OmpA, which is embedded in the outer membrane and reaches down to grasp the peptidoglycan strands. The support provided by the peptidoglycan layer is essential, making it an Achilles' heel of the cell. Many important antibiotics, such as penicillin, kill bacterial cells by attacking the enzymes that build the peptidoglycan layer. When treated with penicillin, bacterial cells lose their shape and ultimately explode under the osmotic pressure.

Along with the peptidoglycan, there are also many small proteins floating around in the periplasm between the two cell membranes. These proteins have first pick of the molecules that enter the cell, and play roles appropriate to this station. The most plentiful ones collect a specific nutrient, like a sugar or an amino acid, and deliver it to the inner membrane for transport inside. The periplasm also contains enzymes that continue the digestion of food molecules, chopping them into small pieces for transport inside. There are also several protective enzymes in the periplasm that detoxify poisonous compounds before they can get any further. These include superoxide dismutase, which breaks down reactive forms of oxygen, and beta-lactamase, which destroys drugs like penicillin.

The inner membrane, or cytoplasmic membrane, is filled with proteins for transport, sensing, energy production, and many other diverse tasks. In its role as part of the protective barrier of the cell, the inner membrane acts as the selective filter of the cell wall. Unlike the outer membrane, it is sealed, preventing random crossing of molecules. Instead, it has many selective pump proteins that locate the desired molecules and transport them inwards or outwards according to need. Some of these, like the calcium pump, require energy from ATP to transport their cargo.

The cytoplasmic membrane also contains several protective devices that fight common dangers. The large multidrug resistance transporter, which spans the entire cell wall, gathers toxic molecules like drugs and poisons and pumps them all the way out of the cell. The tiny mechanosensory MscL protein senses the tension of the cell membrane. If the pressure inside the cell rises too high, this protein opens like an iris and temporarily relieves the pressure.

Building New Proteins

Over half of the molecules inside each *Escherichia coli* cell are involved in one way or another with the synthesis of proteins (Fig. 4.5). In a typical growing cell, 5,000 RNA polymerase enzymes are busy transcribing DNA into RNA, and upwards of 20,000 ribosomes begin making proteins from the RNA strands even before they are finished. The 20 types of transfer RNA molecules circulate around the ribosomes, delivering amino acids for addition to the growing chains. Twenty types of aminoacyl-tRNA synthetase enzymes add new amino acids to the spent transfer RNA molecules. A host of protein factors initiate the process, shepherd each step in synthesis, and terminate the new protein chain when the time is right. Then, chaperonin proteins assist in the folding of the protein. Finally, after the cell is done with the protein, it is destroyed by proteasome molecules like ClpA. Every step in the life of the protein, from start to finish, is carefully controlled by the cell's molecular machinery.

All but a few proteins are encoded in one large circular DNA, about half a millimeter in diameter and containing 4,720,000 base pairs. Bacteria also contain small circles of DNA called plasmids that encode only two or three proteins. Plasmids are easily transferred between bacterial cells and are used to share important genes. For instance, plasmids often encode proteins that attack antibiotics. By transferring the plasmid from cell to cell, resistance to antibiotic treatment can spread through a population of bacteria. Plasmids have also been inordinately useful in biotechnology, making possible an entire industry of genetic engineering.

When bacterial cells are viewed in the microscope, you can see an area in the center that excludes ribosomes, termed the nucleoid. The DNA strands are packed so closely in the nucleoid that they act like a sieve, excluding large molecules. Lots of things are happening there (Fig. 4.6). First, there is a physical problem that must be solved: the half-millimeter circle of DNA must be packaged into a space less than

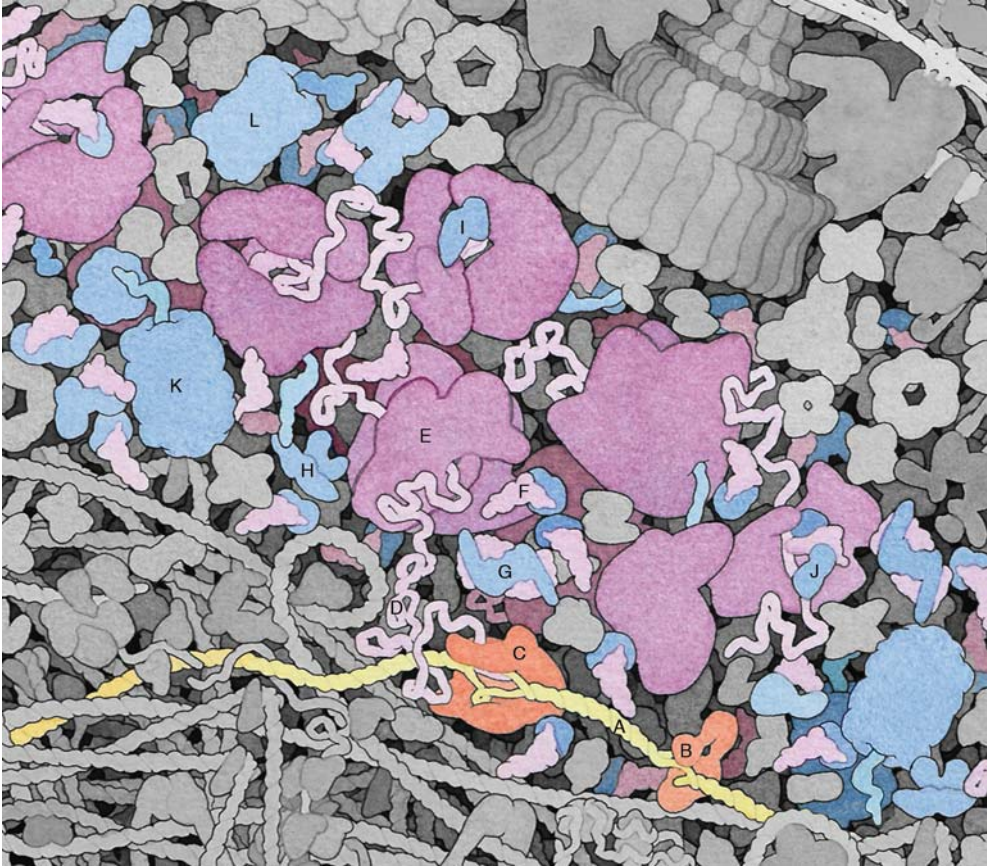


Fig. 4.5 Protein Synthesis in the Bacterial Cell Transcription and translation are performed by a collection of over 50 molecular machines inside the cell. The genomic information is stored in a large circular DNA (A). DNA topoisomerase (B) assists as RNA polymerase (C) unwinds the DNA and builds a messenger RNA (D). Ribosomes (E) then build proteins based on the information in the RNA strand, using amino acid building blocks carried by transfer RNA (F). Aminoacyl tRNA synthetases (G) attach the proper amino acids to the transfer RNA, and the whole process is guided by elongation factors Tu and Ts (H) and elongation factor G (I). Initiation factors (J) get the whole process started by bringing in the first transfer RNA and docking the two subunits of the ribosome. Finally, chaperonins (K) assist with the folding of new proteins, and the proteasome ClpA (L) destroys obsolete proteins

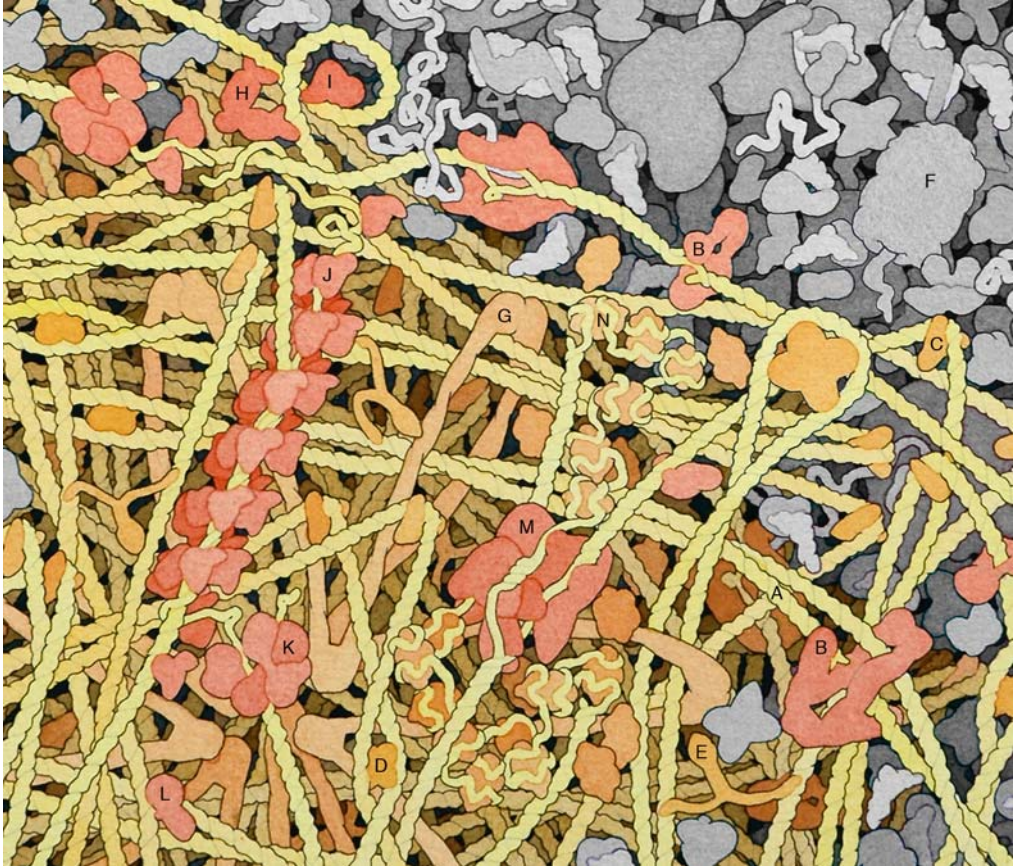


Fig. 4.6 The Nucleoid Proteins in the nucleoid protect, repair, control, and replicate the genetic information stored in DNA. Knots and twists in the large circular DNA (A) are resolved by DNA topoisomerases (B), and proteins such as HU (C), Fis (D), H-NS (E), LRP (F), and SMC (G) help to package the DNA into the small space of the cell. The information in the DNA is controlled by the opposing actions of repressors such as the lac repressor (H) and activators such as the catabolite activator protein (I). Breaks in the DNA are repaired by proteins such as RecA (J) and RecBC (K), and local errors are corrected by enzymes such as MutM (L). DNA polymerase (M) creates new copies of the DNA, with some help from the single-stranded DNA-binding protein (N)

one hundredth its size. Several DNA-packaging proteins, such as HU, fis, H-NS, and LRP, facilitate this process. They bend the DNA, or link two neighboring strands together, or wrap the DNA into tight little bundles. These proteins easily fall off the DNA, however, to allow reading of the information in the DNA.

The tangle of DNA strands also causes some topological problems. The winding and unwinding caused by RNA polymerase can cause unpleasant kinks, and tangled DNA circles must be separated when the cell divides. DNA topoisomerases solve these problems. They make deliberate cuts in the DNA double helix, allowing it to relax or allowing strands to pass by each other. Then, they accurately reconnect the DNA when they are done.

The cell must also control when and where each gene is used. The information held in the DNA genome is highly regulated. A host of repressors and activators interact with each gene, determining when it will be used to create proteins. For example, the lac repressor binds at the beginning of a region that encodes four proteins that are used in lactose metabolism. When lactose is scarce, the lac repressor binds to the DNA and blocks the genes. But when lactose becomes available, it induces a change in the shape of lac repressor, and it falls off. Then the gene is transcribed and the cell makes the proteins needed to utilize the lactose.

Bacterial cells also monitor their genomes for damage, quickly repairing problems if they are found. Many mechanisms are continually at work. The simplest defenses are single enzymes that search for damaged bases. For instance, the MutM protein searches for damaged guanine bases and removes them before they can lead to a mutation in the genome. The cell also has more powerful methods for repairing more extensive damage. One of these mechanisms is performed by the RecABC system. It can repair breaks in the DNA by matching the broken DNA with an intact strand (this is described in more detail in Chapter 7). Of course, this requires an undamaged strand to act as the template for repair. Fortunately, typical *Escherichia coli* cells have several copies of their DNA circle because they are constantly replicating their DNA.

Replication starts at a special site on the DNA circle, which recruits two molecules of DNA polymerase. They start replicating the DNA simultaneously in opposite directions, ending at a site on the opposite side. DNA replication in *Escherichia coli* is very efficient. DNA polymerase adds about 800 new nucleotides every second, taking about 50 min to duplicate the entire circle. However, in a rapidly growing *Escherichia coli* culture, the cells divide every half hour. This doesn't allow enough time to duplicate the entire genome of DNA before each division. *Escherichia coli* cells resolve this problem by starting new DNA duplications before the previous round has finished. When a round of duplication is completed and the two circles fall apart, each new circle is already about half way through the next round of replication. Bacterial cells are truly adapted to exploit their environment!

Powering the Cell

About a fourth of the molecules in each *Escherichia coli* cell are devoted to producing energy (Fig. 4.7). Since *Escherichia coli* bacteria normally live inside our intestines, they have an easy source of energy: they can simply digest some of the food that we eat. Other bacteria, however, live in less hospitable environments and are forced to use all sorts of exotic energy sources, such as the oxidation of sulfur

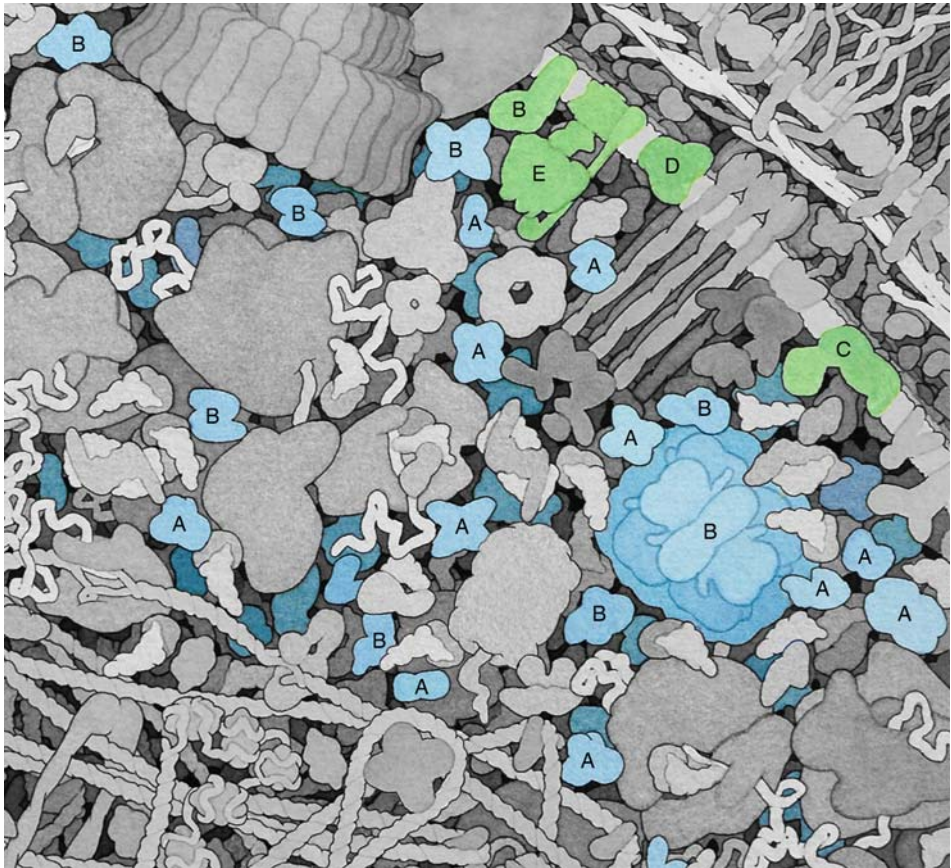


Fig. 4.7 Energy Production The enzymes that capture energy from food are scattered in the inner membrane and in the cytoplasm. These include the enzymes of glycolysis (A) and the citric acid cycle (B). Several of these molecules are involved in the production and use of proton gradients in the inner cell membrane, including the NADH dehydrogenase complex (C), ubiquinol oxidase (D), and ATP synthase (E)

compounds in hot springs. But *Escherichia coli* cells, in general, have it easy. We deliver food directly to them.

The first steps of energy production occur outside the cell, as digestive enzymes are secreted into the surround space. These break down food into manageable pieces that are transported into the cell. Then, the real work begins. *Escherichia coli* cells produce all manner of enzymes that break down food molecules and use the energy to create ATP or electrochemical gradients. Different enzymes are made depending on the food molecules that are available—some specialize in metabolizing glucose, others attack amino acids, others attack fats. These enzymes are mobilized according to need. If the cell stumbles into a particularly rich supply of aspartate, for example, it will build the enzymes needed to assimilate it.

If oxygen is available, *Escherichia coli* cells use a multistep system very similar to our own central system of energy production. It starts with glycolysis, a series of 10 enzymes that take glucose and break it into two pieces. As with all of the cell's chemical transformations, glycolysis is performed in many steps, each under careful control. Two of these steps are particularly energetic, and they are used to create two molecules of ATP. Many organisms stop at this point, using the ATP for energy and discarding the pieces as alcohol (this is how the alcohol in wine and beer is created by yeast).

Our cells and *Escherichia coli* cells, however, add additional steps to squeeze even more energy out of the food molecules. The pieces are completely broken down to carbon dioxide in the citric acid cycle. As with glycolysis, the citric acid cycle is a series of chemical reactions, each one controlled by a dedicated enzyme. As the molecules are broken down, high-energy electrons are captured on several carrier molecules. These electrons are the source of most of our metabolic energy.

In the final step, termed respiration, these electrons flow through a series of proteins like the huge NADH dehydrogenase complex, found in the cytoplasmic membrane. Ultimately, these electrons are placed on oxygen molecules by ubiquinol oxidase, converting them (with the help of a few hydrogen ions) into water. This flow of electrons is a highly energetic process, and it is used to power protein pumps that transfer hydrogen ions across the cell membrane. This creates a powerful electrochemical gradient, which may be used for many useful tasks. For instance, it powers the huge motor that turns the flagella. It also powers ATP synthase, which rotates and creates three molecules of ATP with every turn.

Cellular Propellers

The small size of *Escherichia coli* cells has a profound effect on the way that they interact with their surroundings. For them, gravity is not the overwhelming force that it is in our lives. Instead, the constant press of surrounding water is far more important. At small scales, water is not the flowing liquid that it is to us. The surface tension of water is a familiar example: small insects can skate along the surface of a pond, but if we try, the weight of our enormous bodies overwhelms the gentler forces of water and we plunge to the bottom. For cells, these differences are even greater. Cells live in a world of thick, viscous water, almost oblivious to gravity. When moving from place to place, most of their energy is spent trying to push through the gooey liquid, not in lifting their weight up from the ground.

For example, Howard Berg presented a surprising observation in his 1976 lecture “Life at Low Reynolds Number.” *Escherichia coli* cells swim using long corkscrew-shaped flagella, which act like propellers. The cells push their way through the water, typically moving about 30 $\mu\text{m/s}$ (30 μm is 10 or 15 times the length of the cell). But, when they stop turning the flagella, they don’t keep coasting along the way a ship or submarine would. Instead, the surrounding water instantly stops them in less than the diameter of a water molecule.

The flagellar motor is one of the wonders of the biomolecular world (Fig. 4.8). The motor spans the entire cell wall, rotating at speeds of up to 18,000 revolutions per minute. Each rotation is powered by the flow of about 1000 hydrogen ions across the inner membrane. Amazingly, the motor can turn the flagellum in either direction, clockwise or counterclockwise, on demand. When it turns in one direction, all of the flagella on the cell get tangled into a bundle, and together they propel the cell through the surrounding water. If the motor switches direction, however, the flagella separate and flail in different directions, causing the cell to stop and tumble in place.

Bacteria are faced with a serious problem with navigation. Since they are so tiny, they have no way to aim themselves in the right direction. A bacterial cell can’t look at a distance and see what direction food lies. Instead, *Escherichia coli* cells use an effective combination of the swimming and tumbling properties of their flagella. Each cell uses an array of sensors to determine the level of food in the

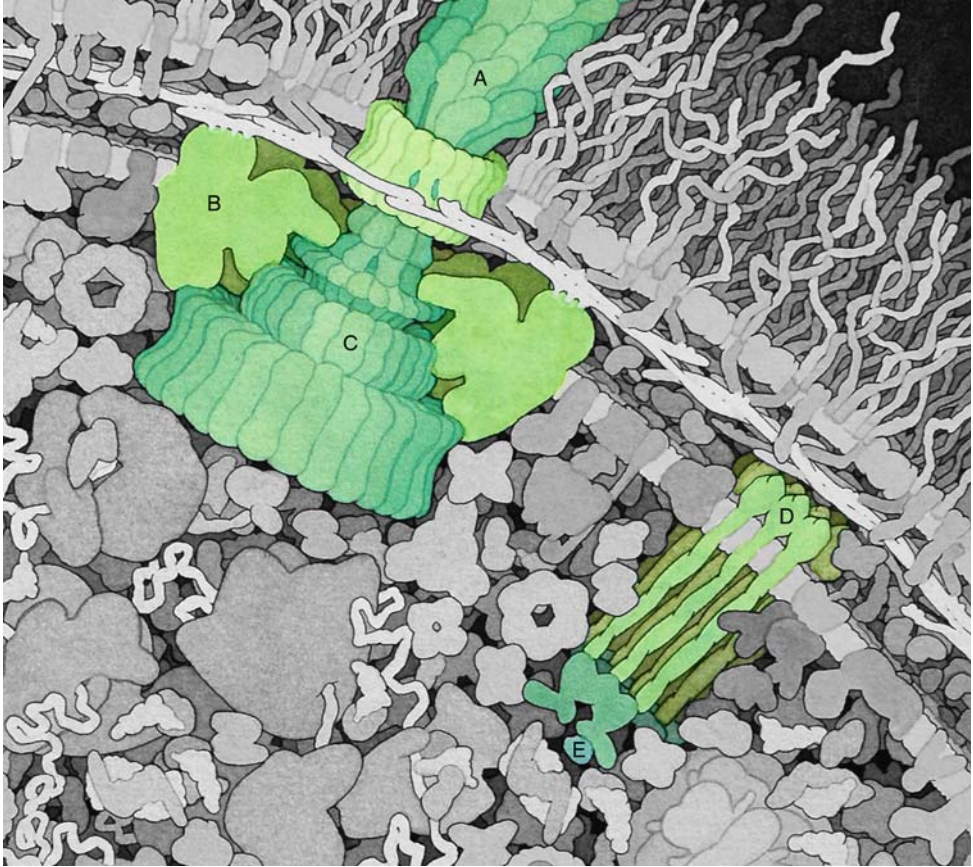


Fig. 4.8 Flagellar Motor The long, corkscrew-shaped flagella (A) are turned by a complex flagellar motor composed of motor proteins (B) and a large cylindrical rotor (C). The motor is controlled by sensor proteins, such as the aspartate receptor (D), that monitor the level of nutrients. When an action is needed, soluble proteins like CheY (E) deliver the signal to the motor

immediate vicinity. Then it swims in a random direction, and measures the level of nutrients there. If the levels are increasing, it keeps swimming in that direction, since things are getting better. If not, the sensory array sends a signal to the flagellar motor, telling it to reverse direction. This causes the cell to tumble, picking a new (and hopefully better) direction to swim. In the rich, watery environment of our intestines, this almost random approach is adequate to keep the cell nourished.

Molecular Warfare

Several hundred different types of bacterial cells, including *Escherichia coli*, live in harmony with us, inhabiting our gut but never causing problems. Over years of evolution, we have negotiated a subtle truce with our bacterial flora. The bacteria have a warm, sheltered place to live and multiply with a consistent supply of food. In return, we obtain several important vitamins from them—molecules like vitamin K and vitamin B₁₂ that are essential for our life, but which our cells cannot make for themselves. The bacteria also assist in the digestion of particularly resilient food molecules, such as some of the tricky carbohydrates in vegetarian foods. In most cases, our relationship with these bacteria is friendly, with both sides gaining something. This is a good thing, since the typical number of bacterial cells in our gut is about 10 times the number of human cells in our whole body!

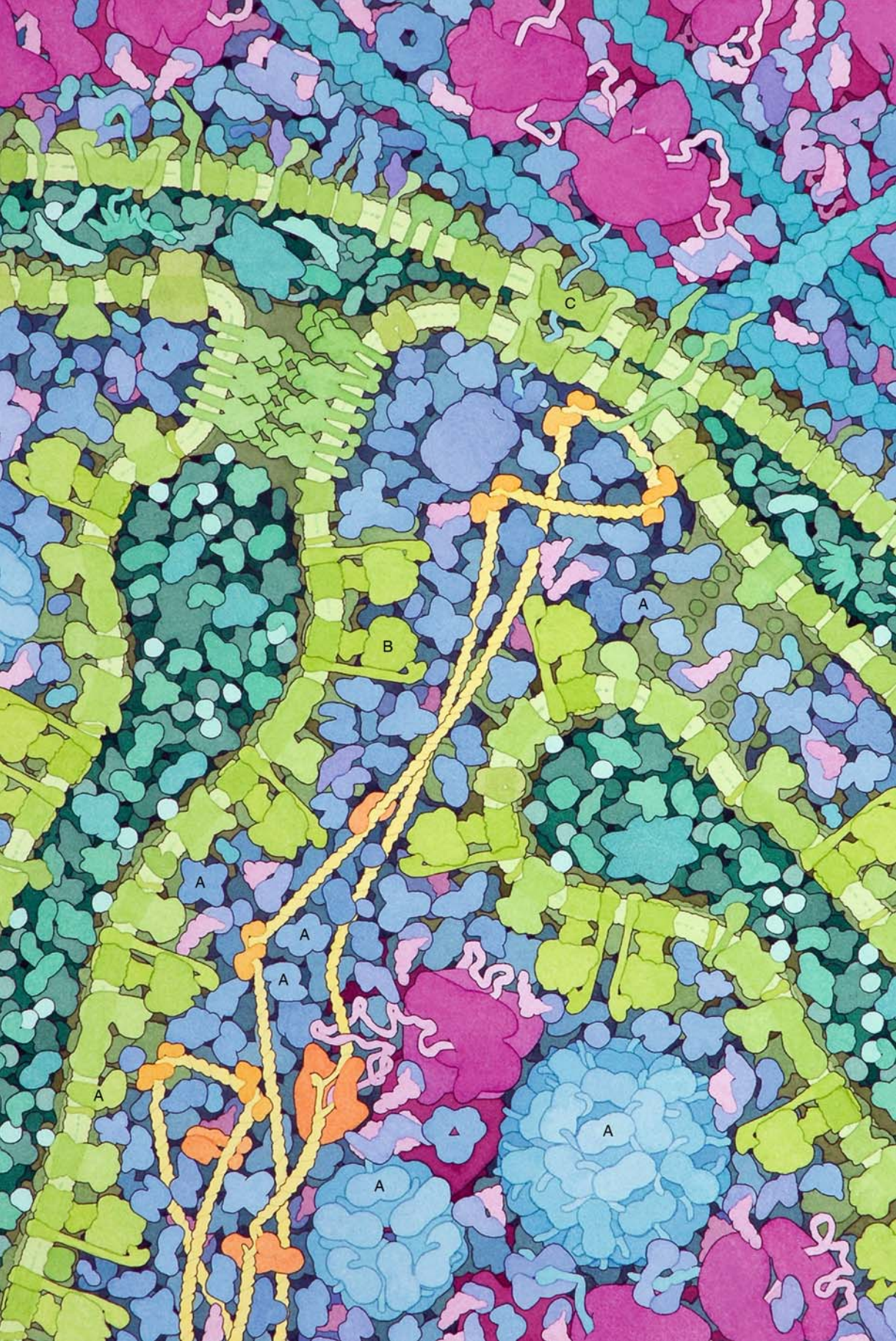
As you might imagine, the intestine is not a perfectly hospitable environment. Bacteria must protect themselves from digestive enzymes and from antibodies, and from being swept away and eliminated from the digestive system. They solve this problem by surrounding themselves with a gluey coat of polysaccharides, forming a biofilm that sticks in place and resists attack by our enzymes and antibodies. They also build long fimbriae and other adhesive proteins that anchor them to the walls. This coating of benign bacteria may be the major advantage of having a healthy crop of intestinal flora. It takes up all the available space and blocks the attachment of pathogenic bacteria, helping to protect us from infection by more dangerous species.

Normally, these cells are quite benign, and even helpful, but they can cause problems if they get into areas where they shouldn't. For instance, if there is an injury, the cells can get through the lining of the intestine and into other parts of the body. Our body has strict defenses against this, standing ready to fight such infections. Our blood is filled with antibodies that recognize bacteria and target them for destruction by the immune system. Our blood also contains an entire system of proteins—the complement system, described in more detail in Chapter 6—that is specifically constructed to find bacterial cells and puncture holes through their cell walls.

Occasionally, *Escherichia coli* cells pick up genes for toxic molecules and turn into pathogenic bacteria. *Escherichia coli* is one of the major causes of traveler's diarrhea, caused by foreign strains of the bacteria that are unfamiliar to the traveler's system. Some strains

create toxins that attack cells directly (described in more detail in Chapter 9). Many of these are destroyed by cooking, so well-cooked meat will reduce the chance of food poisoning by bacteria-tainted meat and produce.

Fortunately, we have powerful drugs to attack these rogue bacteria. These drugs attack different molecules in the bacterial cell, blocking an essential process. For instance, penicillin blocks one of the enzymes that builds the peptidoglycan network. This severely weakens the cells. The bacteria, however, have developed ways to fight back. Drug-resistant strains make an enzyme that breaks down penicillin, protecting the cell from attack. This has led to an escalating biological war, where we develop new antibiotic drugs, and the bacteria find ways to circumvent them.



Chapter 5

A Human Cell: The Advantages of Compartments

The human body is composed of trillions of cells. Just like a bacterial cell, each one of our cells (with a few exceptions) has DNA, polymerases, and ribosomes for building proteins based on genomic information. Each cell is filled with a collection of enzymes for making and breaking the many molecules needed for growth and energy production. Each cell is surrounded by a sturdy cell membrane filled with channels, pumps, and sensors. Each of our cells, however, is much larger and far more complex than a bacterial cell. Where bacteria are built to be fast and efficient, our cells are made to perform complex and specific tasks, and they are made to last and perform these tasks for years.

By looking at the similarities and differences between bacterial cells and animal cells, scientists have discovered that a breakthrough occurred in the evolution of life about 1.5 billion years ago. Simple, bacteria-like cells had probably been around for at least 2 billion years,



Fig. 5.1 Cellular Compartments Unlike simpler bacterial cells, our cells are filled with compartments that perform different duties. ATP synthesis is the primary task performed by the mitochondria, shown here in cross-section. The enzymes of the citric acid cycle (A) are found in the innermost space and the folded inner membrane provides the necessary separation of spaces needed to create the electrochemical gradient that powers ATP synthase (B). This membrane is one of the most protein-concentrated membranes found in our cells: by some estimates, three quarters of the membrane is composed of protein, with just enough lipid to hold it together. Compare this illustration with the illustration of *Escherichia coli* in Fig. 4.1, and notice that the mitochondrion includes very similar machinery for protein synthesis, complete with DNA, RNA, and ribosomes. It does not make all of its own proteins, however, and some must be transported from the cytoplasm by specialized transport proteins (C) in the inner and outer membranes (1,000,000 X)

and most of the basic molecular machinery of life had been perfected. Then, a new design of the cell body appeared: larger cells filled with many small, separate compartments, each surrounded by its own watertight membrane. The proliferation of these new cells signaled a major break in the evolution of cells, and in the years since then, two distinct family lines have evolved. The simple cells, with their molecular machinery jumbled together in one compartment, are the distant ancestors of the bacteria and archaeobacteria. The new compartmented cells gave rise to all other forms of life, including protozoa, fungi, plants, and animals.

Surprisingly, when we look under the microscope, some of these compartments look similar to whole bacterial cells. The mitochondria (Fig. 5.1) in each of our cells have a similar shape, size, and composition as an *Escherichia coli* cell. For instance, mitochondria are surrounded by two membranes. The outer membrane is studded with proteins reminiscent of bacterial porins. The inner membrane, pleated and folded in mitochondria, is filled with proteins of transport and energy production similar to the bacterial proteins. Even more surprising, when you look inside a mitochondrion, it has its own DNA, transfer RNA, and ribosomes, all busily constructing mitochondrial proteins. These remarkable similarities have prompted a theory of the origin of mitochondria (and chloroplasts) that is now widely accepted. It is thought that a bacterial cell took to living inside another cell in the distant past, perhaps entering as a parasite or perhaps surviving the process of being eaten. The bacterium continued to live in this comfortable, sheltered environment, living and reproducing inside the cell. Gradually, both the engulfed cell and the cellular host became increasingly reliant on each other, dividing up the tasks of living. Today, our mitochondria continue to divide and reproduce inside our cells, but they rely on the rest of the cell for many of their essential proteins and nutrients.

Each of our cells is filled with hundreds of compartments. These compartments are a significant advantage in the life of a cell. They allow many extra levels of control in the basic processes of life. Individual tasks, such as protein synthesis or energy production, may be sequestered into a small space, keeping the efficiency of the small bacterial cell. But by adding machinery for directed transport, molecules may be delivered at will from place to place. This allows for a much larger cell, with more direct control of the processes happening from moment to moment, and more sophisticated mechanisms of perception and protection. In the following figures, we will take a short tour through a human cell, exploring these different compartments.

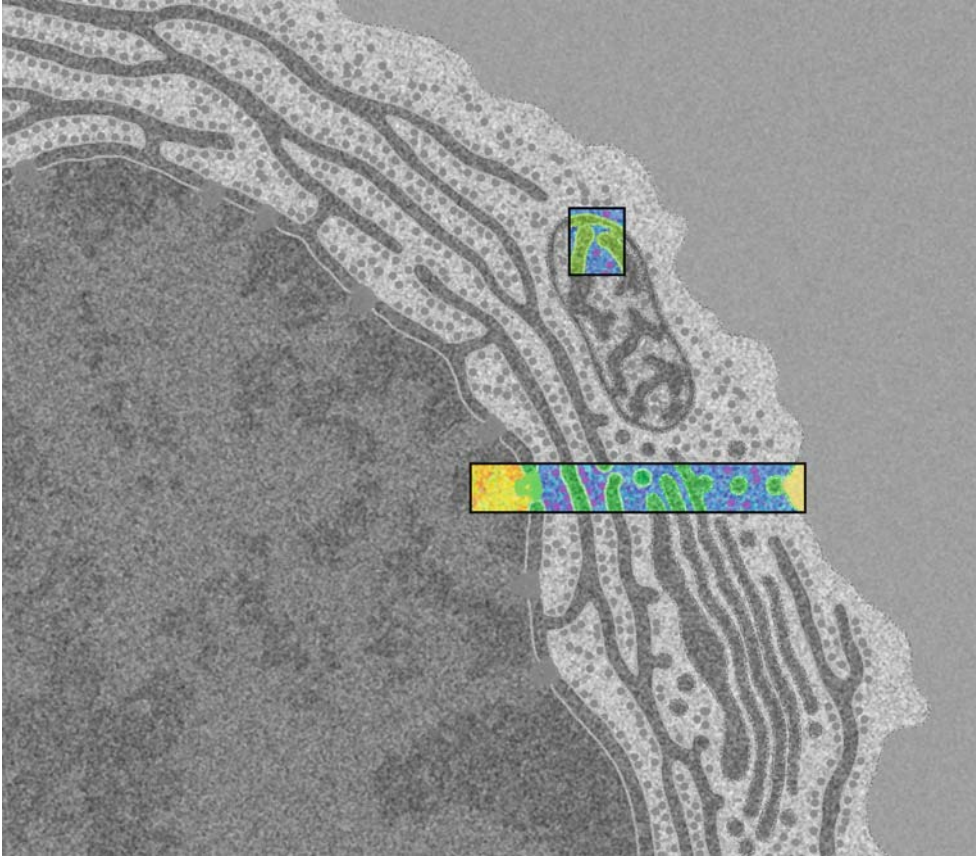


Fig. 5.2 Tour of a Human Cell The boxed regions in this typical human cell, a plasma cell from the blood, are enlarged on the following pages. This cell is dedicated to the production of antibodies, and the tour will follow their synthesis and secretion from the cell. The tour will start at the nucleus where the genetic information is stored, then proceed to the protein production facilities in the endoplasmic reticulum, then move to the sorting station in the Golgi, and finally pass through the cytoplasm to the cell surface. The boxed region at upper left shows the portion of the mitochondrion shown in Fig. 5.1 (10,000 X)

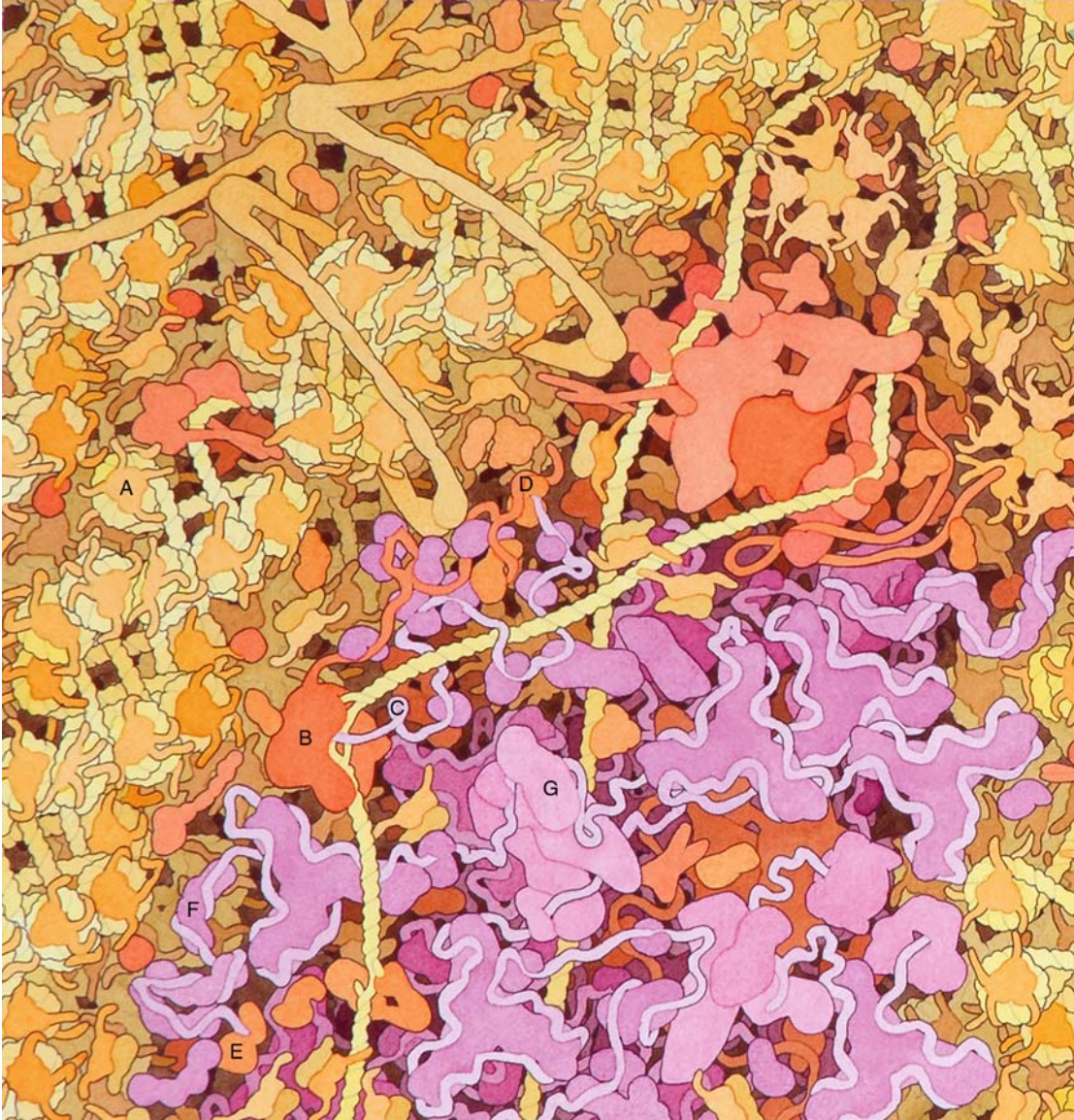
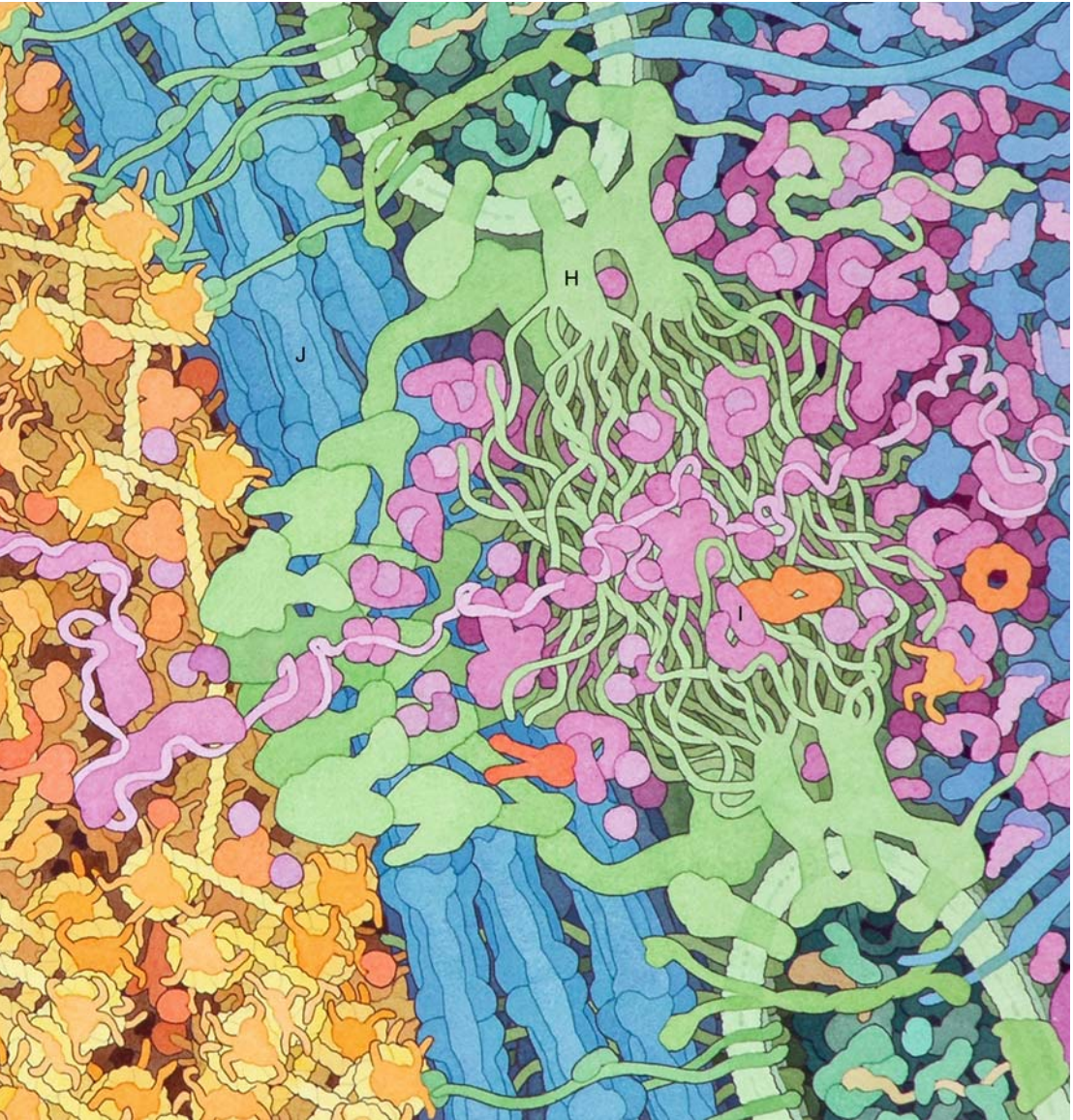


Fig. 5.3 Nucleus The nucleus is the cell's library, storing the delicate strands of DNA and protecting them from the rigors of the cytoplasm. Much of the DNA is wrapped around histone proteins to form small nucleosomes (A) that compact and protect the DNA. When the DNA is needed, it is unwrapped, unwound, and read by RNA polymerase (B) to create a messenger RNA (C). The RNA molecules are then processed: capping enzymes (D) protect one end and polyadenylate polymerase (E) adds a repeated string of adenine nucleotides to the other end, which are then protected by the polyadenylate binding protein (F). Our RNA



must also be edited by large splicosome complexes (G) to remove intron regions that do not encode proteins. Once the RNA is properly edited, it is delivered out of the nucleus through nuclear pore complexes (H). These pores span the double-layered nuclear membrane and control the traffic of a diverse collection of importin proteins (I), which carry other molecules in and out. The nuclear membrane is strengthened inside by criss-crossed layers of lamin protein filaments (J) (1,000,000 X)

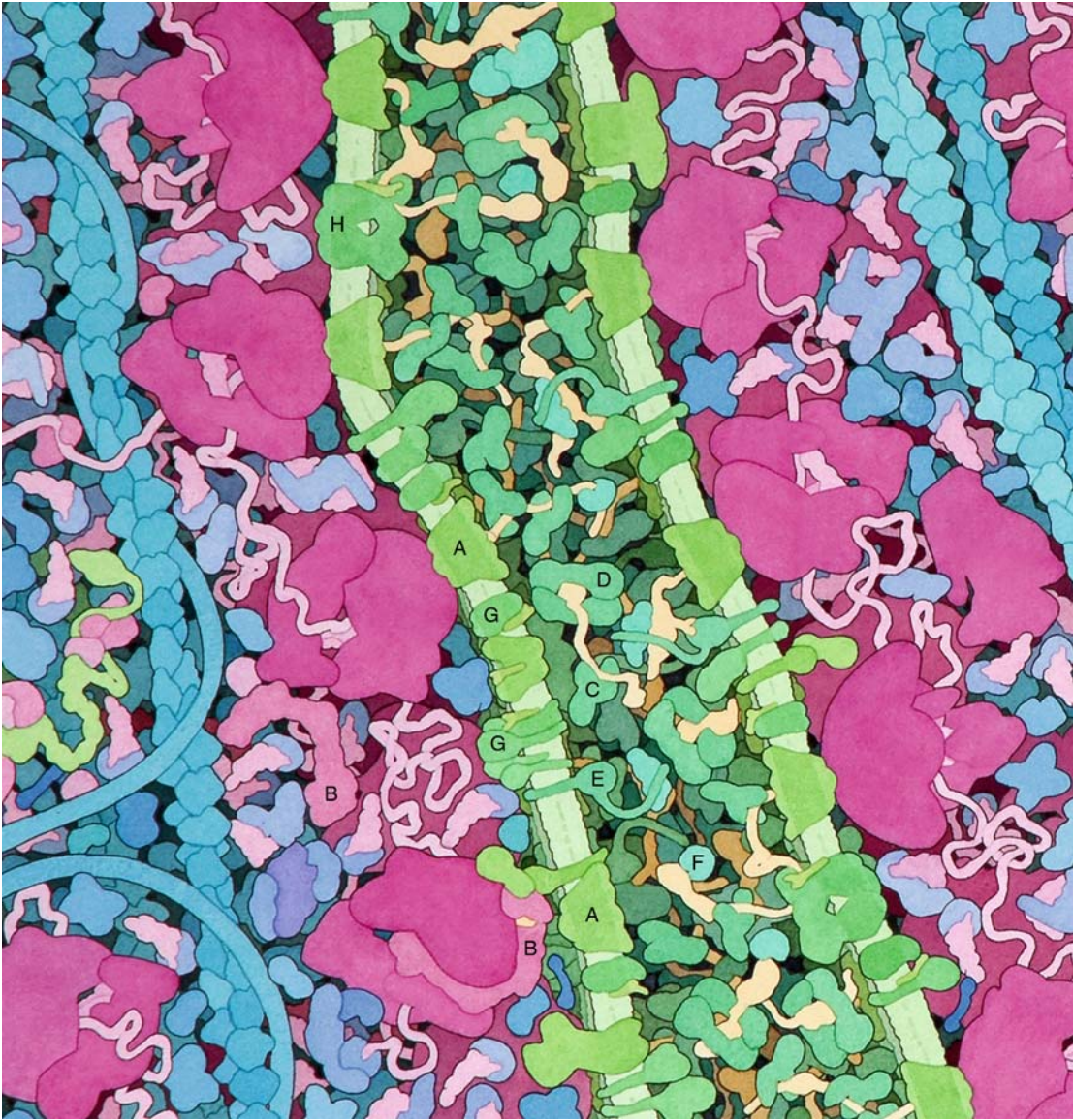
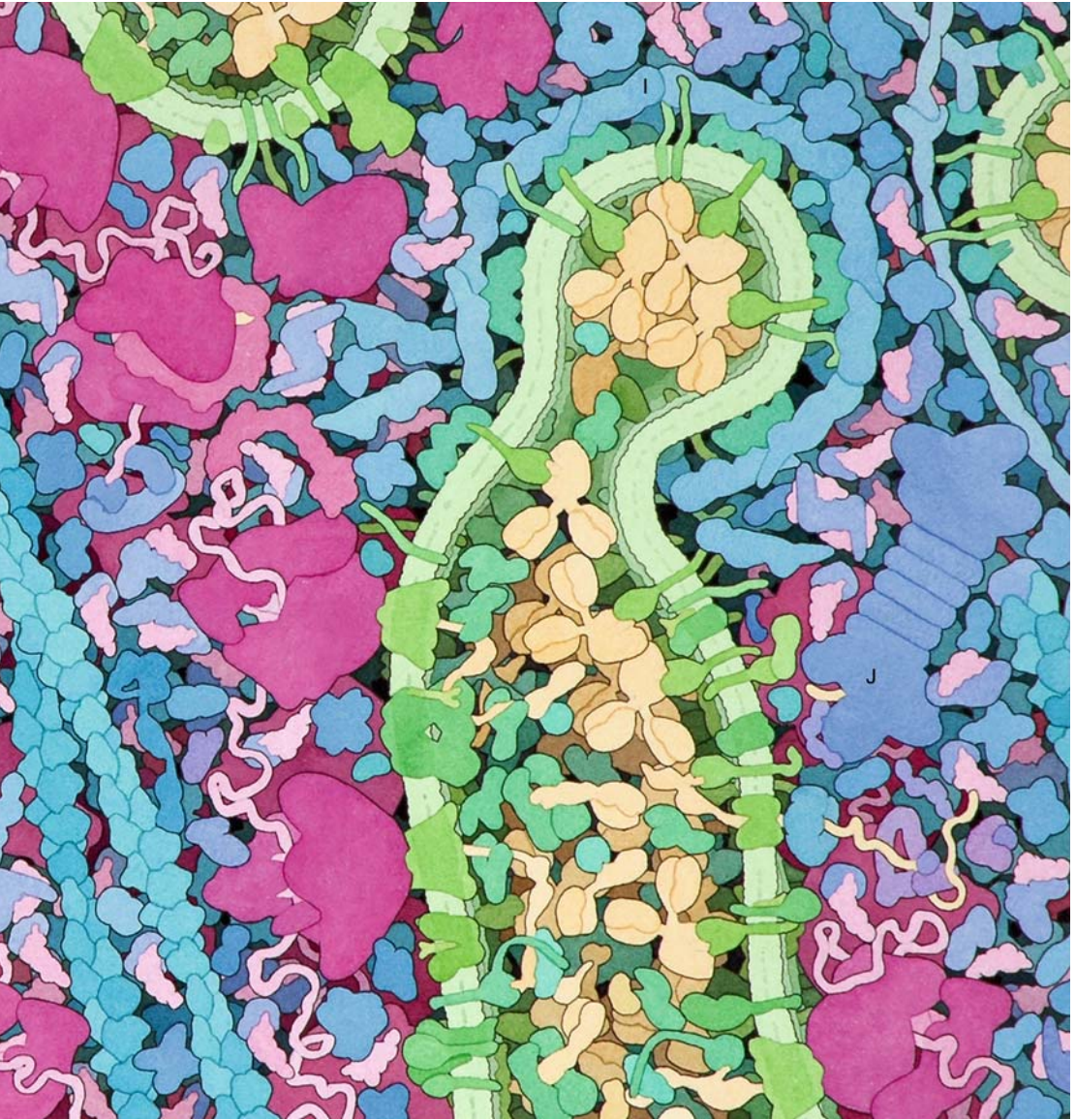


Fig. 5.4 Endoplasmic Reticulum With so many compartments, our cells need ways of sorting and transporting new proteins to their proper destinations. For many proteins, such as the antibodies made by this plasma cell, the journey starts with the endoplasmic reticulum. The endoplasmic reticulum is an interconnected series of tubules and sacs. The membrane surrounding the endoplasmic reticulum is filled with transport proteins (A) that bind to ribosomes and guide new proteins inside as they are being made. The transporter finds new proteins by looking for a special signal sequence of amino acids. This signal sequence is the first thing made by the ribosome, and it is quickly recognized by a signal recognition particle (B) and



delivered to the endoplasmic reticulum surface. Later, the signal sequence is clipped off when the protein is fully synthesized and safely delivered inside. Inside, a collection of chaperonins such as BiP (C), Grp94 (D), calnexin and protein disulfide isomerase (E) and cyclophilin (F) assist in the folding of the new proteins. Polysaccharide chains are made by a series of enzymes in the membrane (G) and finally added to the new proteins by oligosaccharyl transferase (H). Finally, the new proteins are transported to the next step in small transport vesicles, formed by a coat of COPII proteins (I). Any defective proteins are transported out of the endoplasmic reticulum and destroyed by the proteasome (J) (1,000,000 X)

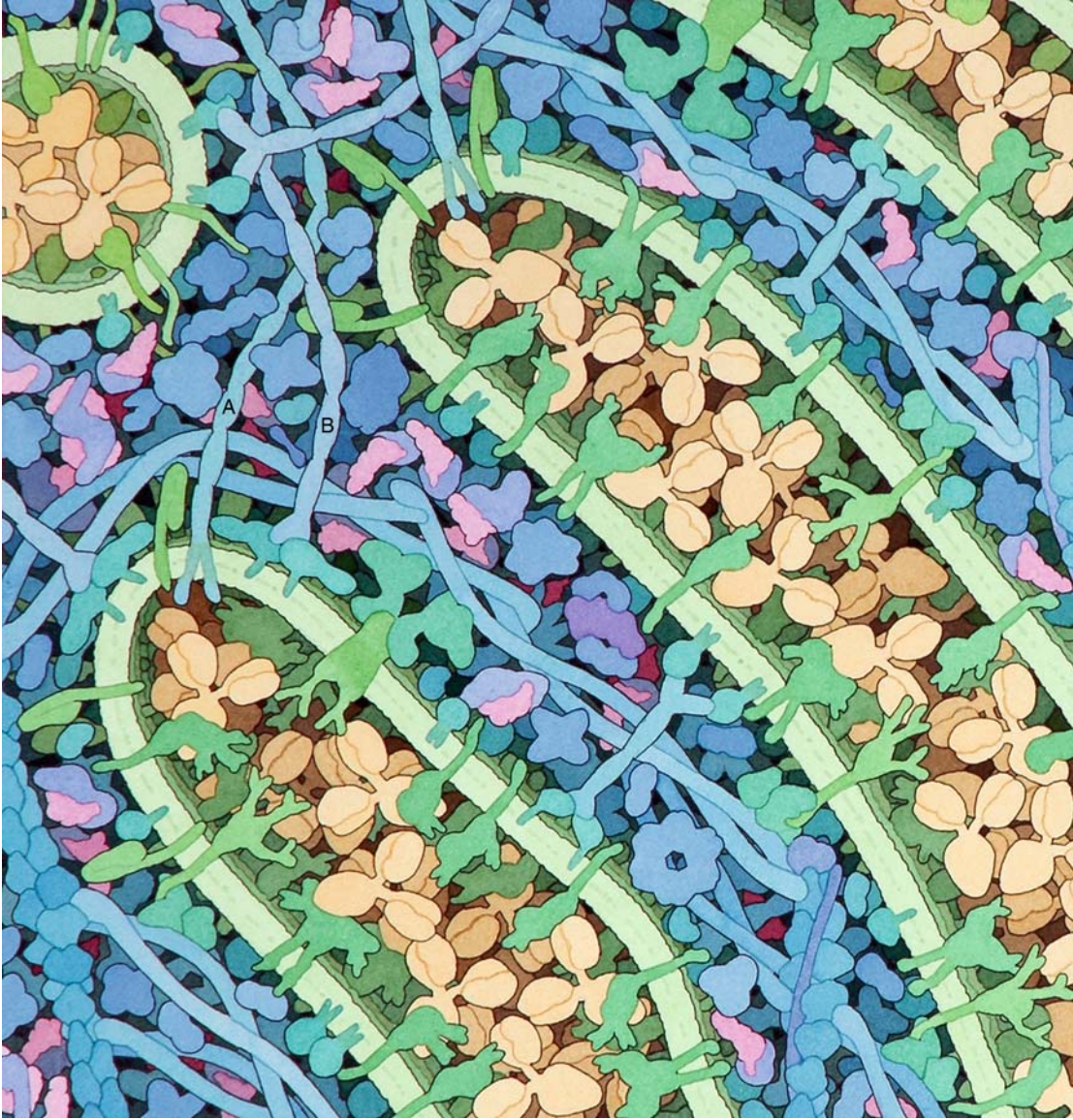
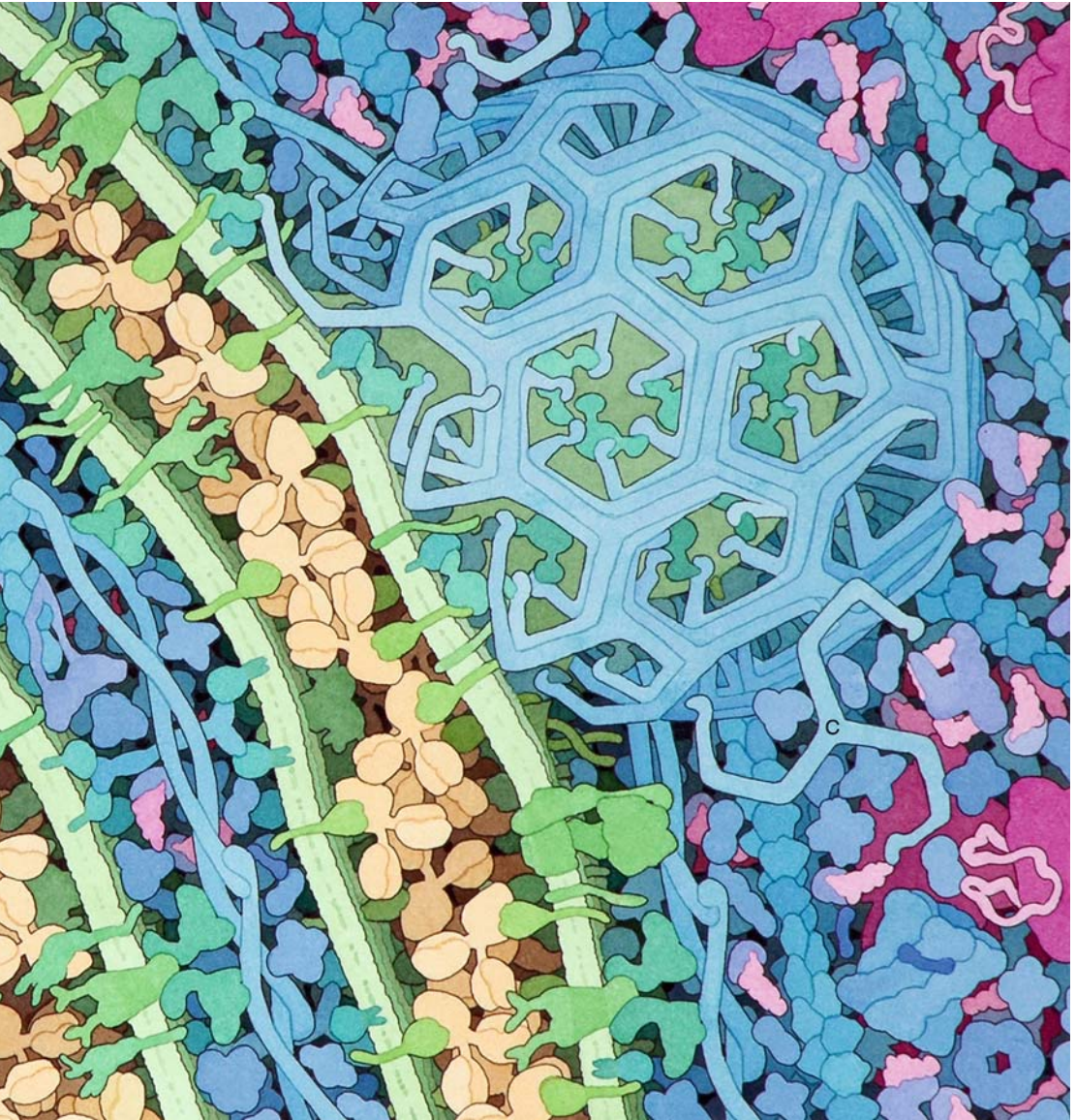


Fig. 5.5 Golgi Apparatus The transport vesicles carry the new proteins to the Golgi, a set of membrane-bounded sacs stacked like plates. Huge tethering proteins like giantin (A) and GM130 (B) guide the vesicles to the right place. The Golgi is the processing and sorting plant of the cell. Sugars and lipids are attached to proteins that need them. For instance, the sugar chains that stabilize the base of the Y-shaped antibody are trimmed and perfected in the Golgi. When



the proteins are properly modified and sorted, they are delivered throughout the cell in small transport vesicles. The protein clathrin (C) provides the molecular leverage needed to pinch off some of these vesicles by forming a geodesic coat on the outside of the membrane. After the vesicle separates from the Golgi, the clathrin coat falls off and the vesicle is guided to its ultimate destination (1,000,000 X)

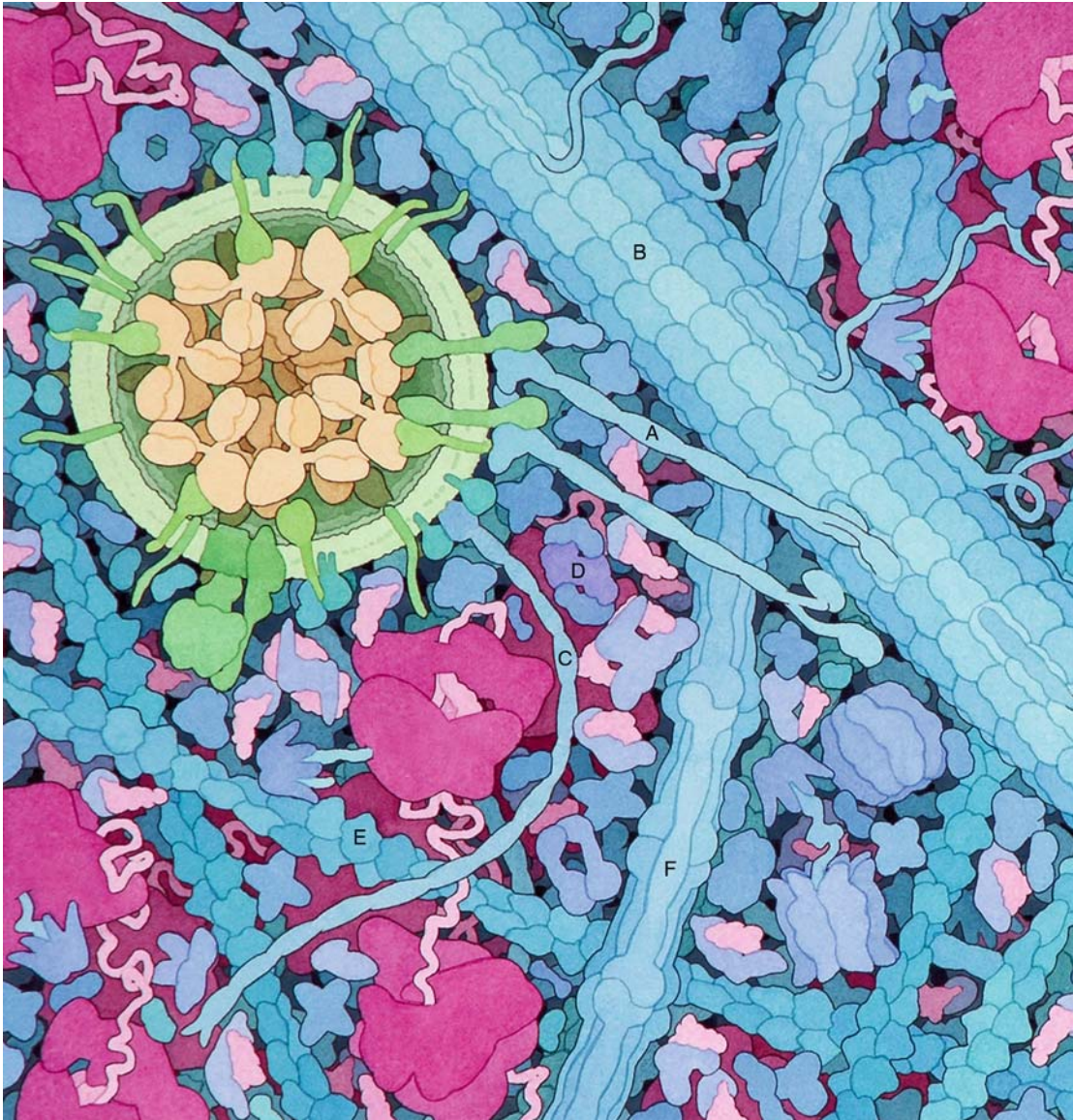
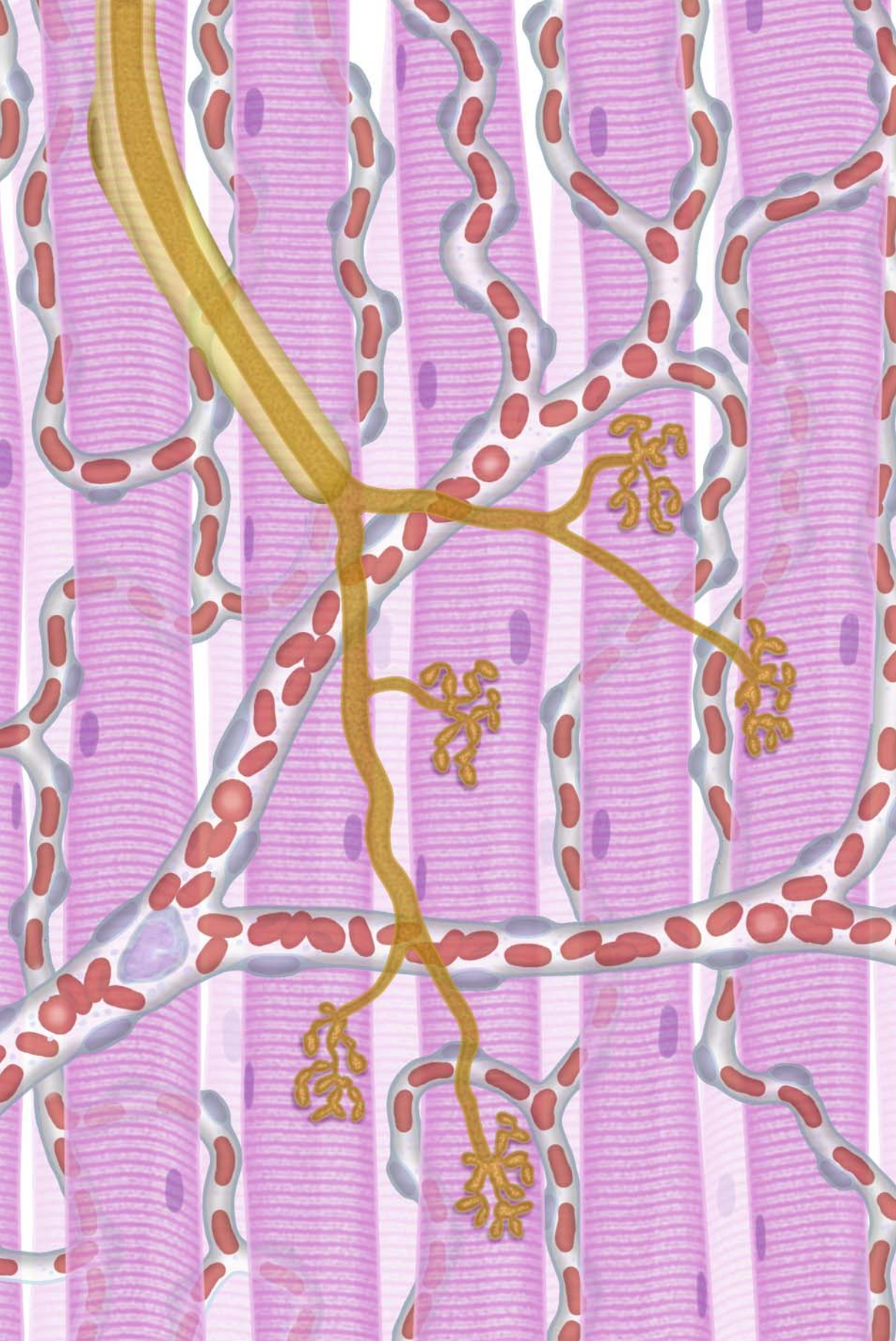


Fig. 5.6 Cytoplasm and Cell Wall The antibodies make their final trip through the cytoplasm to the cell wall, pulled by kinesin (A) along microtubules (B). Long tethering proteins like golgin (C) help the vesicle find its proper destination. The cytoplasm of our cells is filled with enzymes and other proteins performing their many tasks. Many of these are similar to the molecules in bacterial cells, including ribosomes and the other machinery of protein synthesis, enzymes of glycolysis, and other synthetic enzymes. Many new molecules are also present, such as the caspases that will be discussed in Chapter 7 (D). The cytoplasm is criss-crossed with a network of filaments that form a cytoskeleton that supports the cell and provides a railway for delivery of materials. These include thin actin filaments (E), thicker



intermediate filaments (F), and huge microtubules. The cell membrane is studded with a diverse collection of proteins, many of which have polysaccharide groups attached on the outer side. Many of these are involved in traffic of molecules across the membrane and in communication across the membrane. In this plasma cell, there are also some molecules directly involved with its function, such as tethered antibodies (G) used to recognize bacteria and viruses, the IL-4 receptor (H) that receives messages from other cells in the immune system, and SNARE proteins (I) and the exocyst complex (J) that assist the docking and fusion of transport vesicles. On the inside of the membrane, a tough network of spectrin (K) and actin filaments forms a sturdy infrastructure that supports the delicate membrane (1,000,000 X)



Chapter 6

The Human Body: The Advantages of Specialization

Being multicellular has definite advantages. The form and size of the human body, our ability to run and swim, the levels of thought and sensation that we experience. . . all are a consequence of having a body that is composed of many cells. Because we are composed of trillions of individual cells, our cells have the freedom to specialize on their own individual tasks (Fig. 6.1). Cells in our skin specialize in protection by building tough networks of insulating proteins. Cells in our digestive tract are experts in absorption and metabolism of food and nutrients, feeding the rest of the body. Cells in our muscles specialize in generating force, and bone cells entomb themselves in mineral crystals, building a skeleton that articulates this force. A few remarkable cells in the gonads specialize in reproduction. They guard the information and resources that are needed, with the help of a matched cell from the opposite sex, to build an entire new human body. Perhaps most amazing of all, cells in our brain specialize in processing chemical and electrical signals, giving rise to our rich inner world of mind.

In spite of their diversity, however, each of our 10 trillion individual cells closely resembles a simpler single-cell organism like a bacterium. Each contains DNA, polymerases, ribosomes, and everything else needed to make proteins. Each contains the enzymes of glycolysis and



Fig. 6.1 Muscle Tissue This section of muscle tissue contains several highly specialized types of cells, all working together towards the common goal of allowing us to move. These include striped muscle cells that power the motion, bright red blood cells that provide food and oxygen, and branching nerve cells that control the process. There is also a complex infrastructure of connective tissue outside of the cells (not shown here) that supports and shapes the muscle tissue (1,000X)

respiration, allowing them to process sugar into useable chemical energy. Each is bounded by a lipid membrane, separating it from its neighbors and defining its own personal territory. Our cells make their specialized proteins on top of this necessary overhead: lymphocytes make antibodies for export into the blood; nerve cells make chemical sensor proteins and electrical insulation; red blood cells make so much oxygen-carrying hemoglobin that there is not room for much else; muscle cells forge a protein engine composed of actin and myosin.

This diversity creates an informational problem not encountered by simpler organisms. Since all of the cells in the human body are created from a single fertilized egg cell, the single copy of DNA from each parent must hold all of the information needed by every type of cell. Thus, nerve cells carry the information needed to build hemoglobin, and blood cells could conceivably make neurotransmitters. Clearly, however, this must not occur, or the result would be chaos. Each cell must be able to decide which proteins it makes and which ones it ignores, focusing on its role in the blood, brain or elsewhere. This choice is made in the first 9 months of life, as our proliferating cells progressively commit to their different fates. When a cell commits to a function, the need for a host of proteins disappears: developing nerve cells have no need for the hemoglobin genes, and blood cells do not need the information to make myosin. The DNA encoding these unneeded proteins is put into storage and packed in the dusty corners of the nucleus.

A few special cells—stem cells—do not make this commitment. They retain the ability to grow and perform many functions. Embryonic stem cells are the most general of all. They can divide and choose any specialty, becoming skin cells or muscle cells or nerve cells according to their placement in the developing embryo. Adult stem cells typically do not retain this flexibility. Instead, they specialize in generating a particular family of cells. For instance, hematopoietic stem cells in our bone marrow divide and become several types of blood cells, such as red blood cells and white blood cells. These stem cells keep dividing throughout our lives, continually replenishing cells that are worn out or lost.

Infrastructure and Communication

Our cells must deal with structural problems that are far more challenging than those facing small, single cell organisms. To build an organism as large as a human being, elaborate molecular methods of

bracing, buttressing, and connecting cells must be used. The human body is strengthened and shaped at many levels: starting with strong internal bracing of cells, adding methods to weld neighboring cells to one another, and finally using tough membranes and cables to surround tracts of cells and arrange them into tissues, organs, and entire bodies.

Each of our cells is filled with a cytoskeleton that performs the same function as our skeleton of bones: it supports the cell and provides the scaffold for motion. The cytoskeleton is composed of protein filaments, allowing a rare combination of strength and versatility. These filaments are composed of many protein subunits, arranged in long helical cables. When assembled, they may be used in the most strenuous structural tasks, but at the same time, they may be quickly disassembled and reassembled elsewhere to meet the changing needs of the cell.

Actin is the smallest filament in the cytoskeleton, but it is one of the most plentiful proteins in cells, often comprising 5% of the total protein. Actin filaments criss-cross the cell, forming a tangled network that fills and supports the cytoplasm. This network is further strengthened by actin-binding proteins that crosslink the actin filaments. In most cases, this network is not static. For instance, actin filaments play an important role in the crawling of cells. Actin filaments are built subunit-by-subunit at the cell surface, pushing out pseudopods that creep along the ground. When necessary, however, actin can be bundled into strong and permanent girders. For instance, bundles of actin are used to support finger-like protrusions of cells lining the digestive system, and huge arrays of actin are used as part of the engine of muscle contraction (described below).

Two larger filaments assist actin in the cytoskeleton: intermediate filaments and microtubules. Intermediate filaments are more stable than actin. They are composed of interlocking protein building blocks, so they resist disassembly. They are woven through the actin network for strength, and similar filaments of lamin are layered inside the membrane of the nucleus. They also play specialized roles in skin cells and hair, which are composed largely of similar filaments called keratins. These are glued tightly together with chemical crosslinks, forming some of the toughest fibers made by our bodies.

Microtubules, on the other hand, are temporary structures like actin, built when they are needed and disassembled soon after. They act as the railways of the cell. Two types of motor proteins—kinesins and dyneins—drag molecular cargo along microtubules, making deliveries throughout the cell. In nerve cells (described below), they provide the strength to

support their long, thin axons and deliver materials down their amazing lengths, and in sperm cells, dyneins slide microtubules side-by-side to power the swimming. Microtubules also form the beautiful aster-shaped rail system that separates chromosomes when the cell divides.

Most of our cells, however, do not need to crawl around inside our bodies. They remain fixed in tissues and organs, working with their neighbors to perform their function. A variety of junctional molecules hold these cells together and allow them to communicate. For instance, arrays of cadherin proteins reach between neighboring cells and form a sturdy adherens junction that glues cells together (Fig. 6.2). These proteins also extend into the cell, binding to actin filaments inside. In this way, the junction links the cytoskeletons of the two cells, strengthening entire tissues.

These types of cell-to-cell connections are fine for small regions of tissue, but to build large structures like muscles and organs, stronger materials are needed. These structures are built outside cells, surrounding and supporting them. They have many physical forms, ranging from elastic fibers to materials tough as concrete that last for centuries. Some tissues, like cartilage and bone, are mainly composed of this structural material, with only a scattering of cells acting as caretakers. Other tissues, like the densely packed neurons in the brain, have just enough to hold the cells together.

The building blocks of this extracellular matrix are built inside cells, then exported out of the cell and assembled in place. The major component of the extracellular matrix is collagen—it is so common that roughly one-fourth of the protein in your body is collagen. Collagens come in many forms, but all are built around a core of three protein strands wound tightly together like the strands in a rope. Several types of collagen are built for different purposes. One is a long, almost featureless strand that associates side-by-side to form huge collagen fibrils that provide most of the strength of the extracellular materials. Other collagens have specialized structures attached at each end of the strand, allowing them to form extended net-like structures. The networks are then woven together with other proteins and polysaccharides to create a tough basal lamina that surrounds and shapes cells (Fig. 6.3). Another collagen sews collagen fibrils and the basal lamina together.

Along with these structural challenges, our cells must also have ways to communicate with one other, to ensure that they are coordinated and working towards a common goal. Neighboring cells in tissues communicate directly with one another by physically linking

their cytoplasm together. Gap junctions (Fig. 6.2) are constructed in regions where the cell membranes of two neighboring cells come into close contact. Each of the connexon proteins in the gap junction has a tiny pore connecting the two cells. Normally, the gap junction watches over a busy traffic of small molecules back and forth between the cells, but they can be closed in emergencies. For instance, if the calcium level of one cell rises sharply—often a signal that the cell is sick or damaged—the connexons snap closed and quarantine the unhealthy neighbor.

Messages are also passed between cells in a local neighborhood using molecular messengers, termed cytokines. These are small proteins that are built by one cell and then dropped outside the cell. They diffuse to a neighbor and are captured by receptors on the cell surface. This triggers a signal inside the cell, causing it to take appropriate action. A constant dialogue of cytokines allows cells to discuss the current state of the tissue, determining if it is time to grow or time to rest. Cytokines are also used to warn of danger. For instance, cells build alpha-interferon as a warning that there may be viruses in the vicinity. It tells cells to build specialized virus-fighting molecules, such as nuclease enzymes that attack viral RNA, helping them protect themselves until the immune system can be fully mobilized to fight the infection. Similarly, messages may be sent over even larger distances by dropping a molecular messenger into the blood. As described in more detail below, hormones carry messages to cells throughout the body through the bloodstream.

Using these basic tools of ultrastructure and communication, the human body coordinates the action of about a hundred different types of cells. For the rest of this chapter, we will explore three tissues—muscle, blood, and nerves—and see some of the specializations that are used for their particular tasks.

Muscle

All of the motions that you are making right now—holding this book in your hands, scanning your eyes across the page, holding your back and neck steady as you sit—are powered by myosin. Myosin is a tiny molecular muscle that uses the chemical energy in ATP to perform a deliberate motion. As it breaks a molecule of ATP, it performs a forcible bending motion. One of these “power strokes” is enough to move a molecule a few nanometers, but when it is combined with many

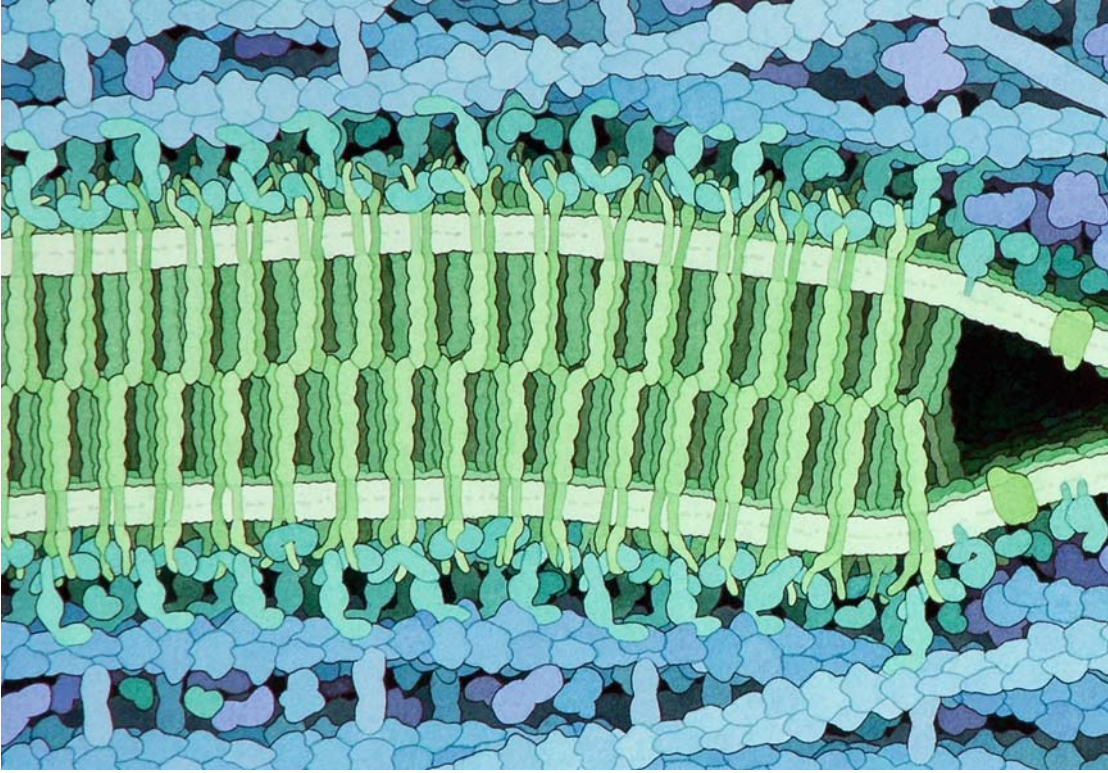
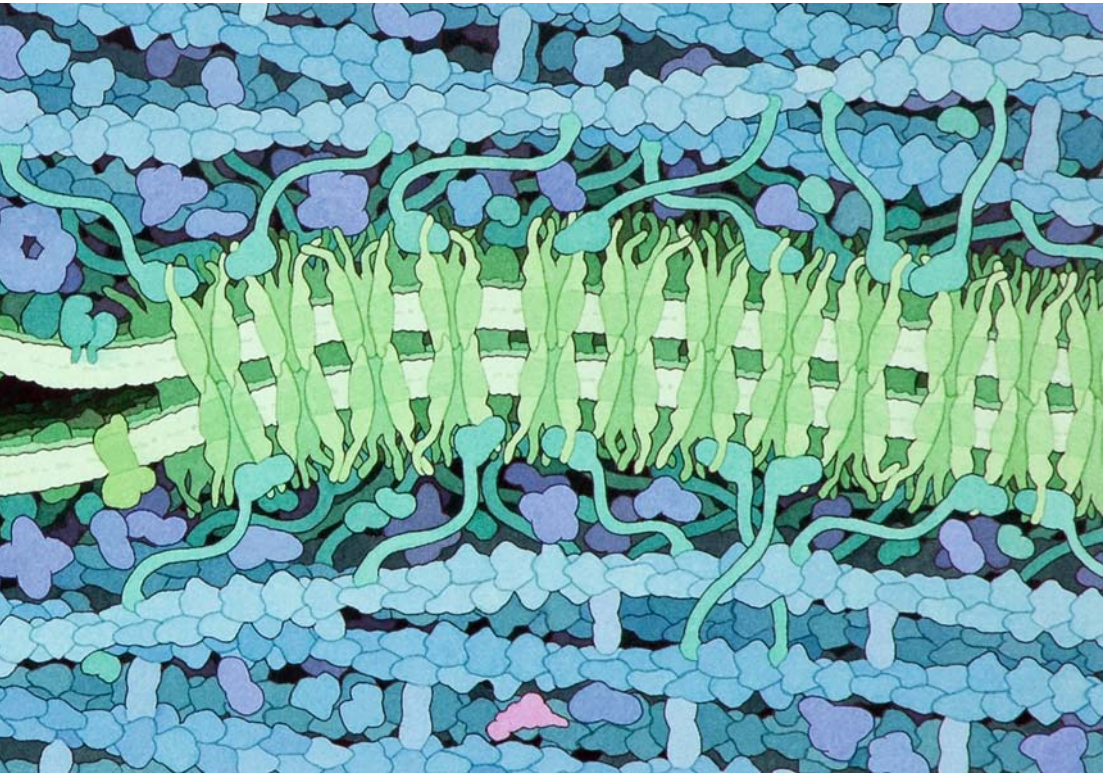


Fig. 6.2 Cellular Junctions Two types of cellular junctions are shown. On the left, many cadherin molecules (*in green*) reach across the space separating the two cell membranes to form a sturdy adherens junction. On the inside of the cell, each



cadherin is connected to the cytoskeleton by small adapter proteins. On the right, an array of connexon proteins (*in green*) forms a molecule-sized tube connecting the two cells in a gap junction (1,000,000 X)

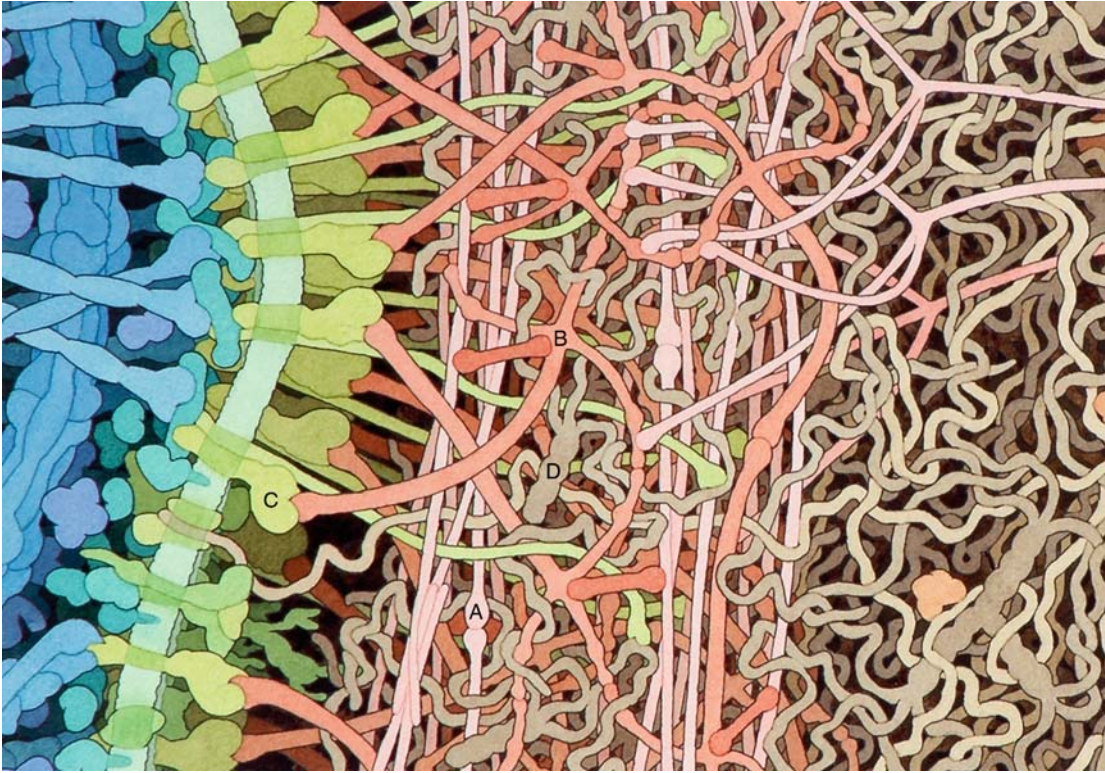
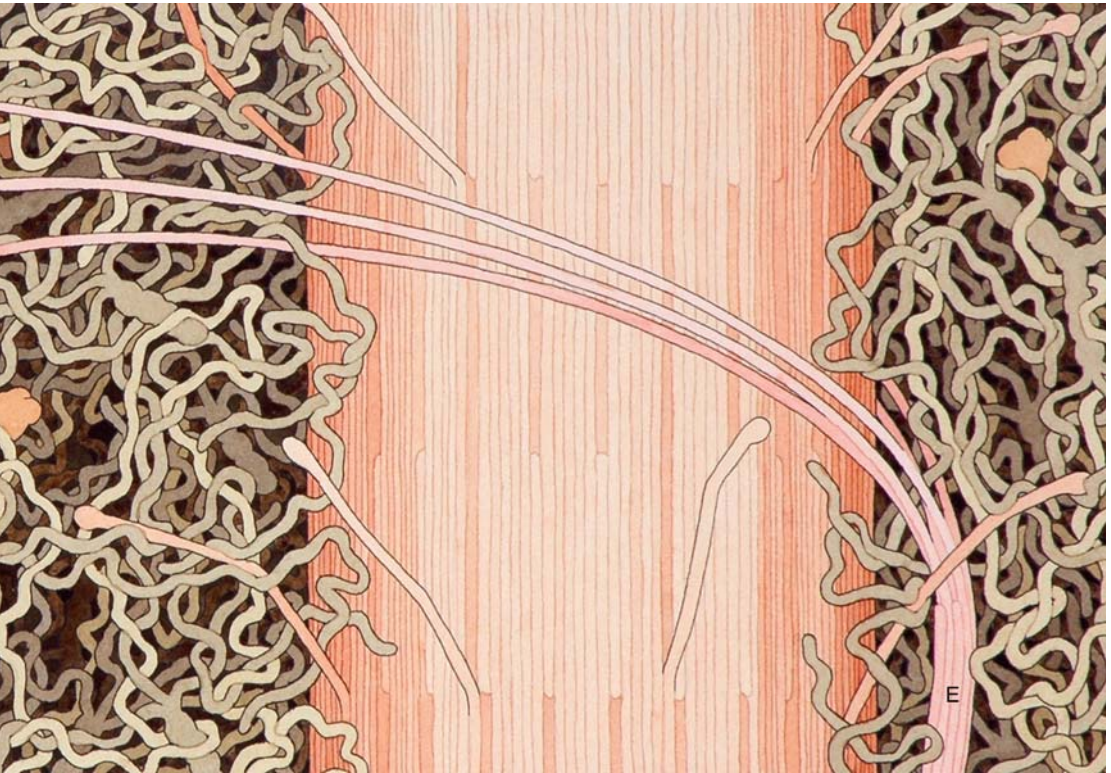


Fig. 6.3 Extracellular Matrix The extracellular matrix is composed of a tangled network of structural proteins and polysaccharides. In this illustration, a cell is shown at far left in cross-section, a dense basal lamina is to the right of the cell membrane, and a collagen fibril takes up most of the opposite page. The basal lamina is composed of molecules such as collagen (A) and cross-shaped laminin (B). Integrin proteins (C) in the cell membrane link the basal lamina to the



cytoskeleton inside the cell. Large proteoglycans (D) are woven into the basal lamina and mixed with long polysaccharide chains in the space outside. Several different types of collagen are shown in this illustration, including the collagen network in the basal lamina, another form that makes large anchoring fibrils (E), and several types that assemble together to form the huge structural fibril (1,000,000 X)

other myosin molecules (10^{19} in our larger muscles) all stroking together, it can lift your entire body.

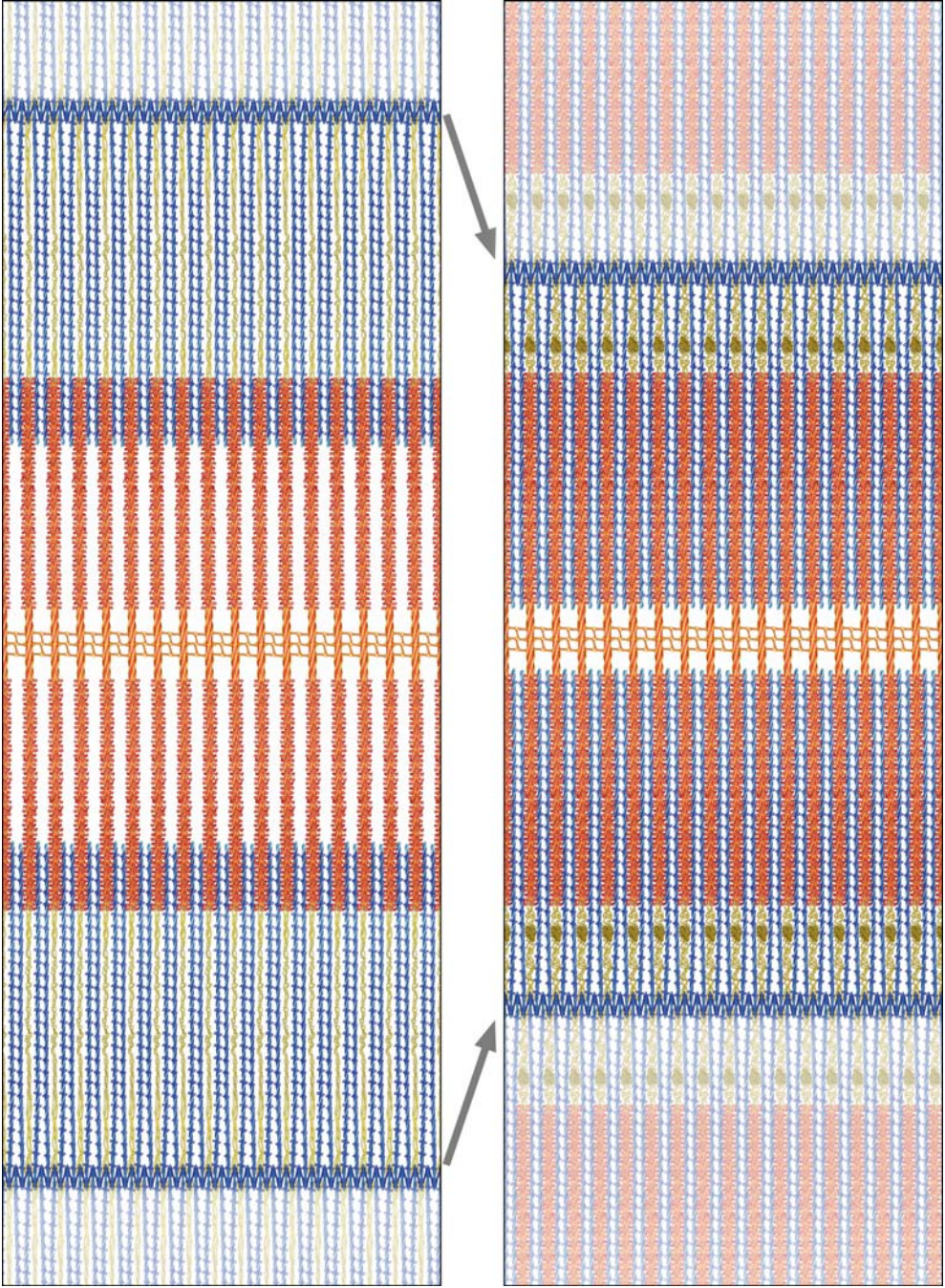
Our muscle cells are filled with sarcomeres, molecular engines composed of myosin and actin (Figs. 6.4 and 6.5). Sarcomeres combine the tiny motions of myosin molecules to create large-scale motions. Within each sarcomere, actin and myosin filaments are arranged side-by-side, and the many myosin molecules crawl their way along the actin. Each sarcomere can contract by about a micron—about 60% of its length—but when 10,000 sarcomeres are stacked on top of one another in a long muscle cell, it can contract by enough to move your arm.

Since so much force is being generated, each sarcomere needs to be structurally sound. The actin filaments are all stitched together by a network of proteins at the end of each sarcomere, and the myosin filaments are stitched together by a second network of proteins at their center. The whole thing is integrated by the huge protein titin, which acts like a rubber band connecting the actin apparatus to the myosin apparatus. Since it is stretchy, it does not get in the way of the contraction and expansion, but it is limiting enough that it keeps the filaments in their proper alignment, ensuring that the whole engine runs smoothly.

Each sarcomere must be carefully controlled so that the contraction occurs smoothly along the entire muscle fiber. Muscle contraction is controlled by the level of calcium in the muscle cell. It is stored in special compartments and released when the cell needs to contract. The calcium quickly diffuses through all the sarcomeres, triggering a change in troponin molecules that are bound to the actin. This uncovers the sites for myosin binding, starting a wave of contraction. When it is finished, calcium pump proteins transport the calcium back into storage, and the cell relaxes. The whole process is remarkably fast—think of your beating heart, and this repeating cycle of calcium release and storage that occurs every second, continuously for decades.

Blood

Blood is our waterway of transport and communication, and our major line of defense against injury and infection (Fig. 6.6). We have about 5 or 6 liters of blood circulating through our body. About 45% of this blood is composed of red blood cells, filled with oxygen-carrying hemoglobin. There are also a variety of white blood cells, about one for every 700 red blood cells, that prowl around the circulatory system



protecting us from infection (an example was shown in the last chapter). The remaining volume is blood plasma, a concentrated solution of proteins and small fragments of cells that performs many vital functions.

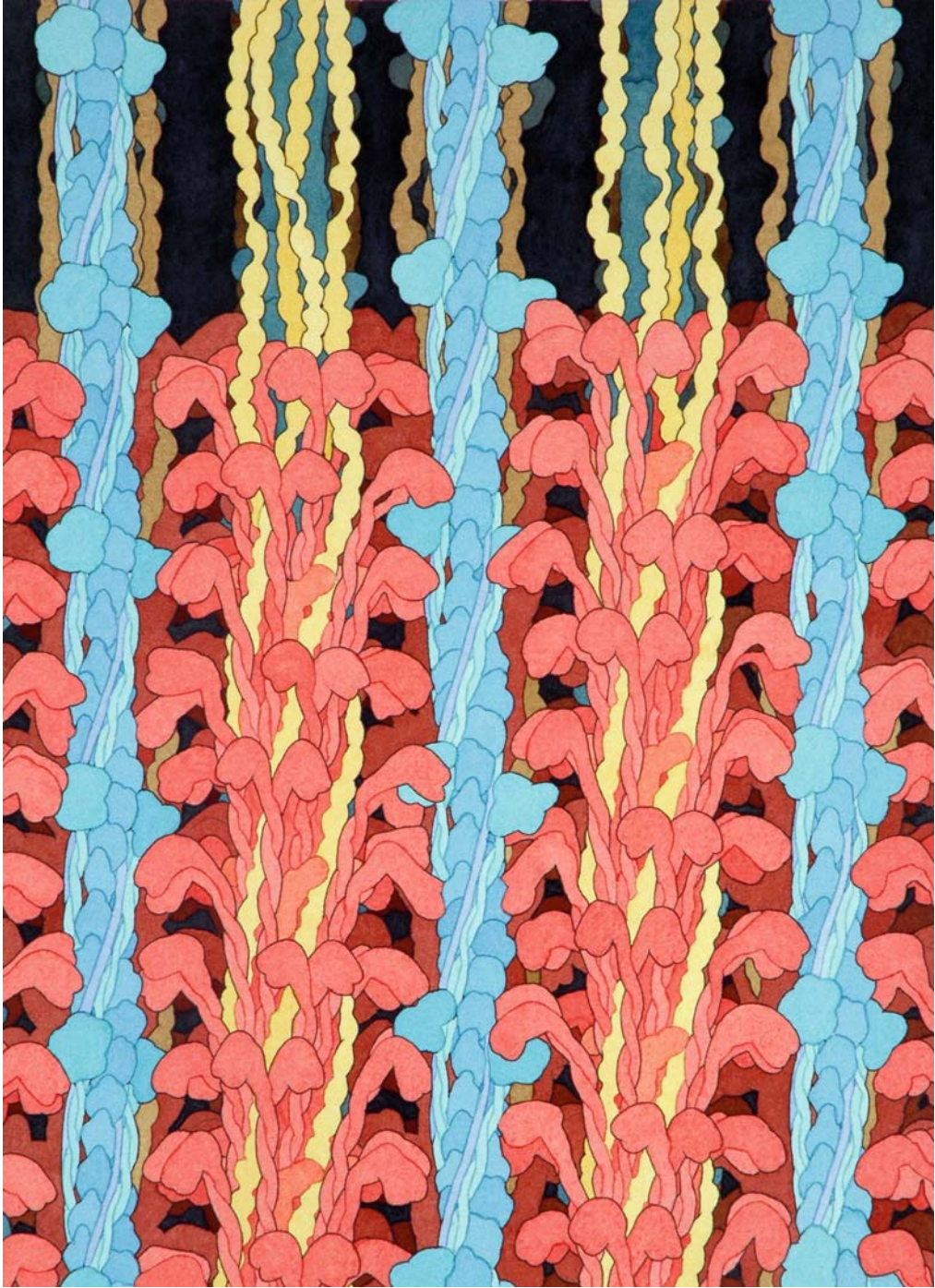
The bright red color of blood is due to hemoglobin carried in red blood cells. Red blood cells are unselfishly dedicated to their work of carrying oxygen from the lungs to the tissues. In fact, they can do little else. Red blood cells are created from stem cells in the bone marrow. As they develop, they gradually shift their resources almost entirely to the building of hemoglobin, and allow all of their other functions to atrophy. The cell membrane loses much of its machinery for communication and selective transport, and is braced only by a rudimentary scaffolding that helps the cell hold its distinctive disk-like shape. Finally, the cell makes the ultimate sacrifice. It concentrates all of its normal molecular machinery—mitochondria, nucleus, ribosomes—into one corner and ejects it all from its body. The mature red blood cell, now a directionless automaton, is then placed into the bloodstream where it carries oxygen through the blood for about 4 months.

Oxygen is not the only molecule transported in the blood. The liquid component of blood, the plasma, is filled with a diverse collection of transport proteins that pick up molecules in one place and deliver them elsewhere. The major problem faced by the blood serum is the transport of fats and lipids. Sugars, since they are so soluble in water, may be simply dumped into one part of the circulatory system and then picked up by hungry cells elsewhere. Lipids and fats, on the other hand, aggregate in a watery environment. If they were put directly into the bloodstream, they would build up and block circulation like grease in a clogged kitchen drain (this is approximately what happens in atherosclerosis). Instead, lipids and fats must be chaperoned on their journey through the blood.

Fatty acids (the carbon chains in fats and lipids) are transported in serum albumin, the most plentiful protein in blood serum. Each albumin molecule can carry seven fatty acid molecules. They bind in deep



Fig. 6.4 Sarcomere Contraction Sarcomeres are composed of myosin filaments, shown here at the center in *red*, interdigitated with actin filaments, shown in *blue*. The many myosin motors on each filament climb along the actin filaments, causing the sarcomere to contract. The thin snaky molecule shown in *yellow* is titin, the huge, stretchy protein that keeps the actin and myosin filaments in proper alignment



crevasses on the protein surface, sheltered away from the surrounding water. Serum albumin also binds and delivers other carbon-rich molecules such as steroid hormones and drugs. This can be very important when deciding on the proper dosage for a given drug. If it binds strongly to serum albumin, most of it will be hidden away in the protein and won't get to its proper site of action. However, it can also prolong the action of a drug. Serum albumin creates a long-lasting reservoir of drug, which is slowly released as it continues to circulate through the blood. Serum albumin also protects drug molecules from our natural mechanisms of detoxification, so they will last even longer than a water-soluble drug.

Lipids and fats are carried a different way. Instead of carrying them one by one inside a protein like serum albumin, they are carried in small globules called lipoproteins. Each lipoprotein is composed of a collection of fat or lipid molecules surrounded by a ring of protein. These lipoproteins are absorbed by cells lining the circulatory system and disassembled inside. There are two major types of lipoproteins circulating in the blood: low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Low-density lipoproteins are larger and contain more lipids, thus giving them the lower density. Both forms are important for the transport of cholesterol around the body. LDL, however, has gotten a bad reputation as "bad cholesterol," since it has a tendency to build up on the walls of arteries that feed the heart and the brain, leading to atherosclerosis. High levels of HDL, on the other hand, appear to correlate with a reduced risk of heart disease, and have been dubbed "good cholesterol." The exact mechanism of this protection is still a matter of controversy, but it may be due to the ability of HDL to transport of cholesterol away from plaques and back into storage in the liver.

Blood serum also contains an internal repair system. Since blood is a liquid resource pumped at high pressure through the body, it is easily lost if the circulatory system is injured. Damage is controlled by the formation of blood clots, which dam leaks until the surrounding tissue can be rebuilt (Fig. 6.7). Clots are formed of long, thin fibrinogen



Fig. 6.5 Actin and Myosin The force of muscle contraction is generated by the many myosin motors extending from the myosin filament (red). Powered by ATP, they reach over and pull the actin (blue) one tiny step forward. Tropomyosin molecules, wrapped around the actin filament, control the process (1,000,000 X)

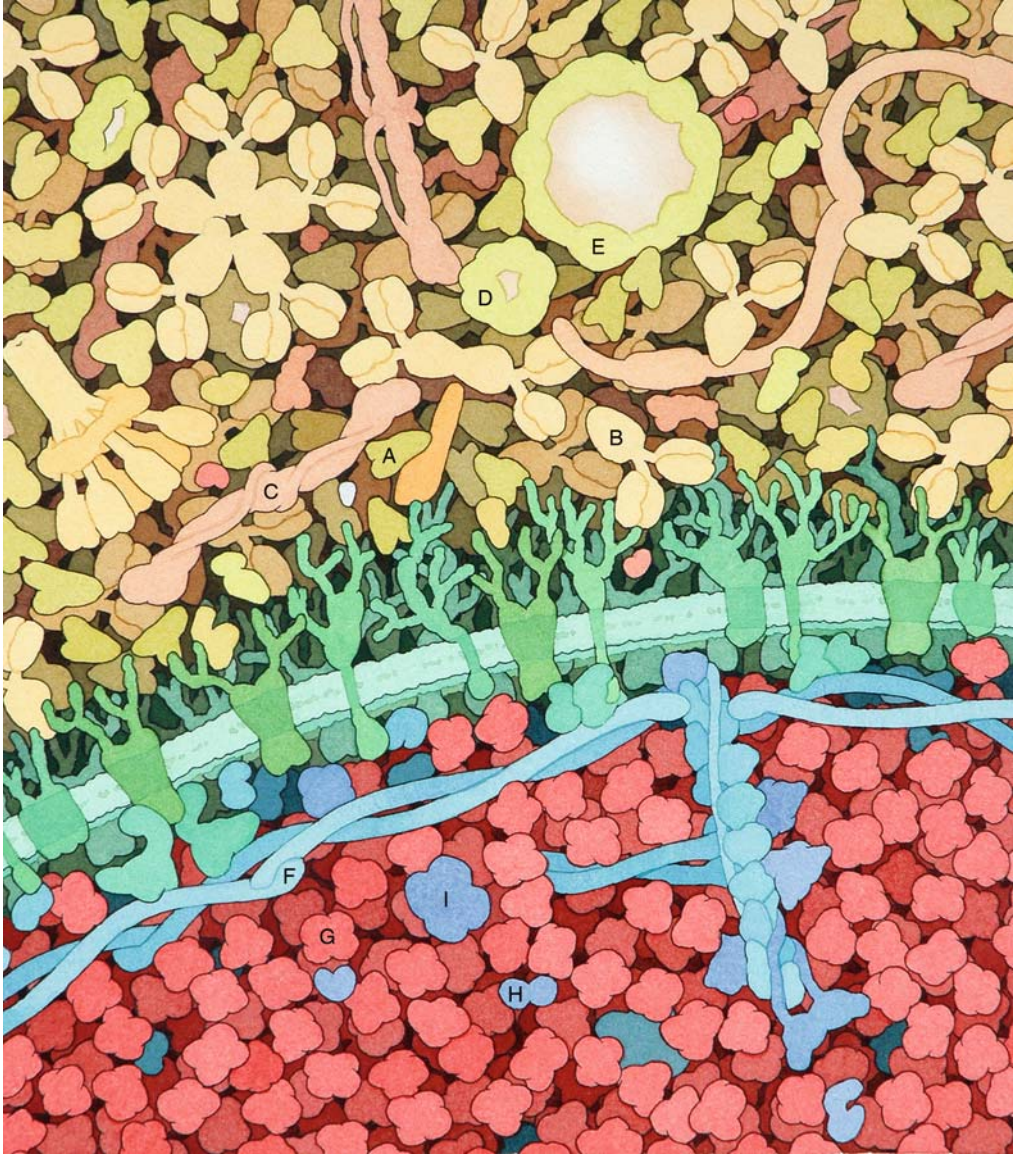


Fig. 6.6 Blood Plasma and a Red Blood Cell This illustration shows blood plasma at the top, and a cross-section through a red blood cell at the bottom. The plasma includes many serum albumin molecules (A), Y-shaped antibodies (B), fibrinogen (C), high-density lipoprotein (D), low-density lipoprotein (E), and many other proteins involved in transport and protection. The cell membrane of the red blood cells is highly simplified compared to the cell membranes of most of our cells, with a simplified spectrin network (F). Inside, the cell is filled almost entirely with hemoglobin (G) and a few antioxidant proteins like superoxide dismutase (H) and catalase (I) (1,000,000 X)

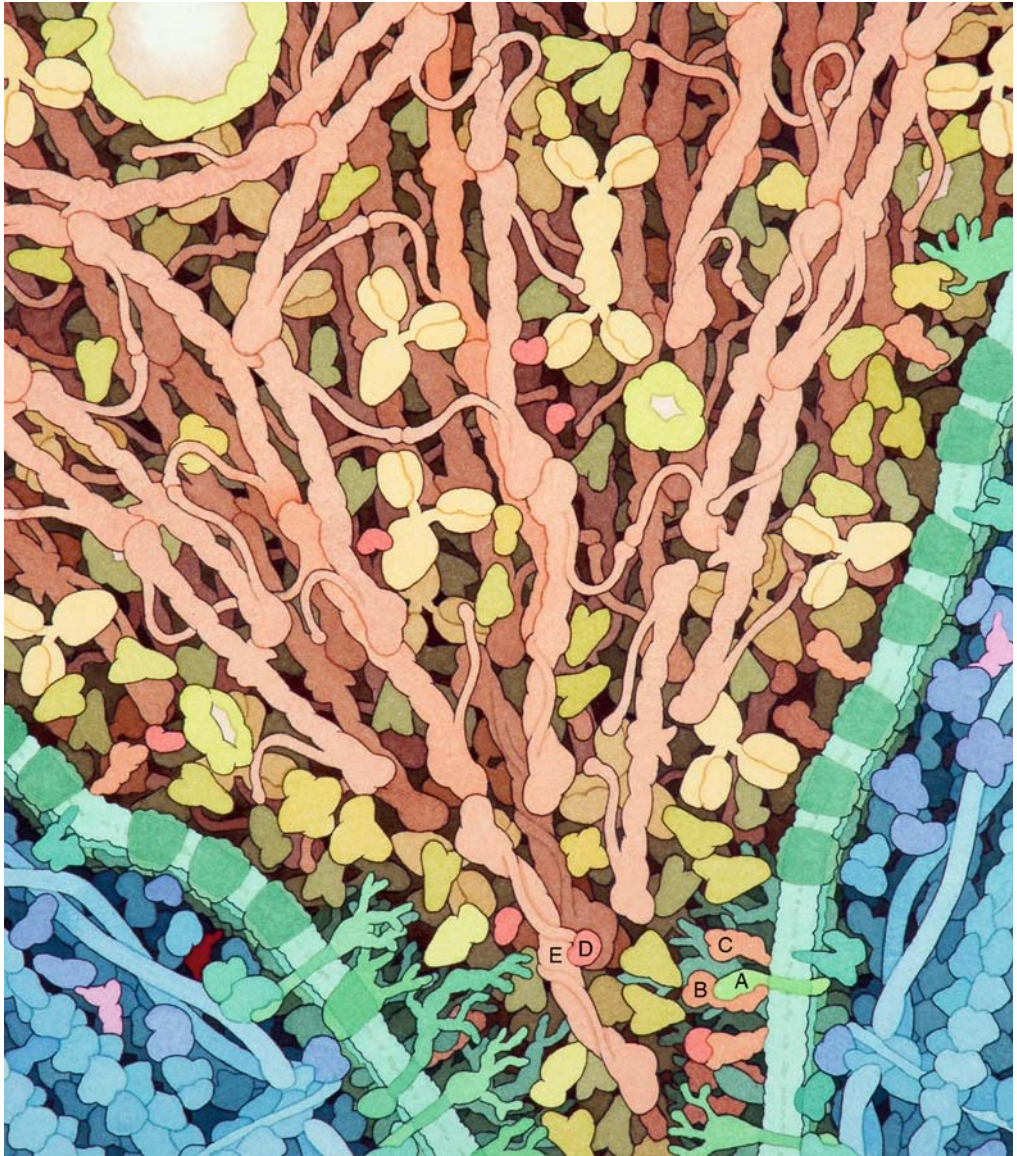


Fig. 6.7 Blood Clotting Blood clotting is initiated when the tissue factor protein (A) is exposed to blood. This initiates a cascade of activation of other blood factors, such as Factor VII (B), Factor X (C), and thrombin (D). Thrombin ultimately activates fibrin (E), which assembles into a sturdy network, shown at the top (1,000,000 X)

molecules, which associate to form a tangled network of filaments, and small cellular fragments called platelets, which get stuck in the net and form a gooey plug that blocks the injury. As you might imagine, this process must be delicately controlled. Blood must only clot at exactly the right time and place, otherwise it would cause blockages that lead to heart attacks or strokes. Blood clotting is controlled by a cascade of proteins, which allow clots to be formed quickly and precisely in the proper location.

The blood-clotting cascade begins with the tissue factor, a protein found on the surface of cells that surround blood vessels, but not on cells normally in direct contact with blood. It is the signal that a tissue has been injured: if the blood reaches a cell that has tissue factor, that means that the blood has leaked out of the vessel, signaling a breach. The cascade then begins. Tissue factor activates a few copies of Factor VII, which then activates many copies of Factor X, which then activates even more copies of thrombin, which finally makes a little clip in many, many copies of fibrinogen, forming the watertight clot. Two tricks are used to create the perfect clot. First, this cascade amplifies the signal. At each step, more and more copies of the proteins are activated, amplifying the signal from a few tissue factor molecules into thousands of activated fibrin molecules. Second, each of these proteins has a very short lifespan. They are highly unstable once they are activated, so they can diffuse only a short distance from the site of injury, creating a localized clot in the proper place.

Much of the machinery of our immune system is also found in the blood, providing constant protection from invading organisms like viruses and bacteria. Antibodies in blood plasma are our first line of defense. They locate the unfamiliar molecules attached to foreign organisms, and tag them for destruction by white blood cells. The plasma is teeming with different antibodies, each tailored to bind to a different non-human molecule. You might wonder how antibody-producing cells design an antibody that will bind to a protein that the body has never seen. As with much of biology, this is done by a combination of randomness with selection. Early in our development, the growing immune system uses a tricky recombination of genes to create many, many different lymphocytes. Each one makes a unique type of antibody. Some of these will bind to our normal proteins, and some will bind to other proteins that we have never encountered. Then, the immune system culls all the cells that make antibodies against our own molecules, so the cells that survive into adulthood only make a collection of random antibodies that bind to foreign molecules.

Mature lymphocytes also have the ability to make slight modifications in these antibodies once they are proven useful, changing an amino acid here and there to perfect its function against new perils.

Blood plasma contains another collection of immune proteins—the complement system—that specializes in killing bacteria. It is particularly important to fight bacterial infections in the blood, since bacteria could quickly reach all parts of the body if they gain a foothold there. The complement system is a cascade of proteins similar to the blood-clotting cascade, with a sensor that activates several rounds of amplification proteins, which ultimately arm a bacteria-killing warhead. The sensor is a six-armed protein called C1. When several of these arms bind at the same time to antibodies on a bacterial surface, it triggers the destructive cascade. Ultimately, this leads to the formation of membrane attack complexes that pierce holes through the invader's cell wall (illustrated as the frontispiece).

Blood is also our simplest mode for communication between cells. Hormones carry messages one-by-one through the blood. Some are small molecules composed of only a few atoms, and others are small proteins. They are created in special glands and received by receptors on and in cells throughout the body. For instance, adrenaline (epinephrine) is created by the adrenal glands (on each kidney) and carries a message that tells cells to focus their effort on energy production. It is often released when we are in immediate danger: the “rush” we feel when faced with a perilous situation is our cells mobilizing their energy resources to ready us for action. Insulin and glucagon are small protein hormones that carry updates about the level of sugar in the blood, stimulating cells to take sugar from the blood or add more. Modified forms of cholesterol are our sex-specific hormones: estrogens made in the ovaries or testosterone made in the testes send the message that it is time to start making changes during puberty. Growth hormone, a small protein made in the pituitary gland, helps coordinate the growth of cells throughout the body during childhood.

Many of our most ancient messages are carried by hormones, orchestrating the underlying rhythms of multicellular life. The use of hormones as messengers, however, is limited to these basic messages, because hormones are so costly to develop. For each new message, an entirely different molecule must be created, as well as a new set of molecular machinery to receive it at the target cells. Today, hormones are used to send simple messages like “I am hungry” or “I am scared,” and a much more flexible and powerful system is used to send more complex messages.

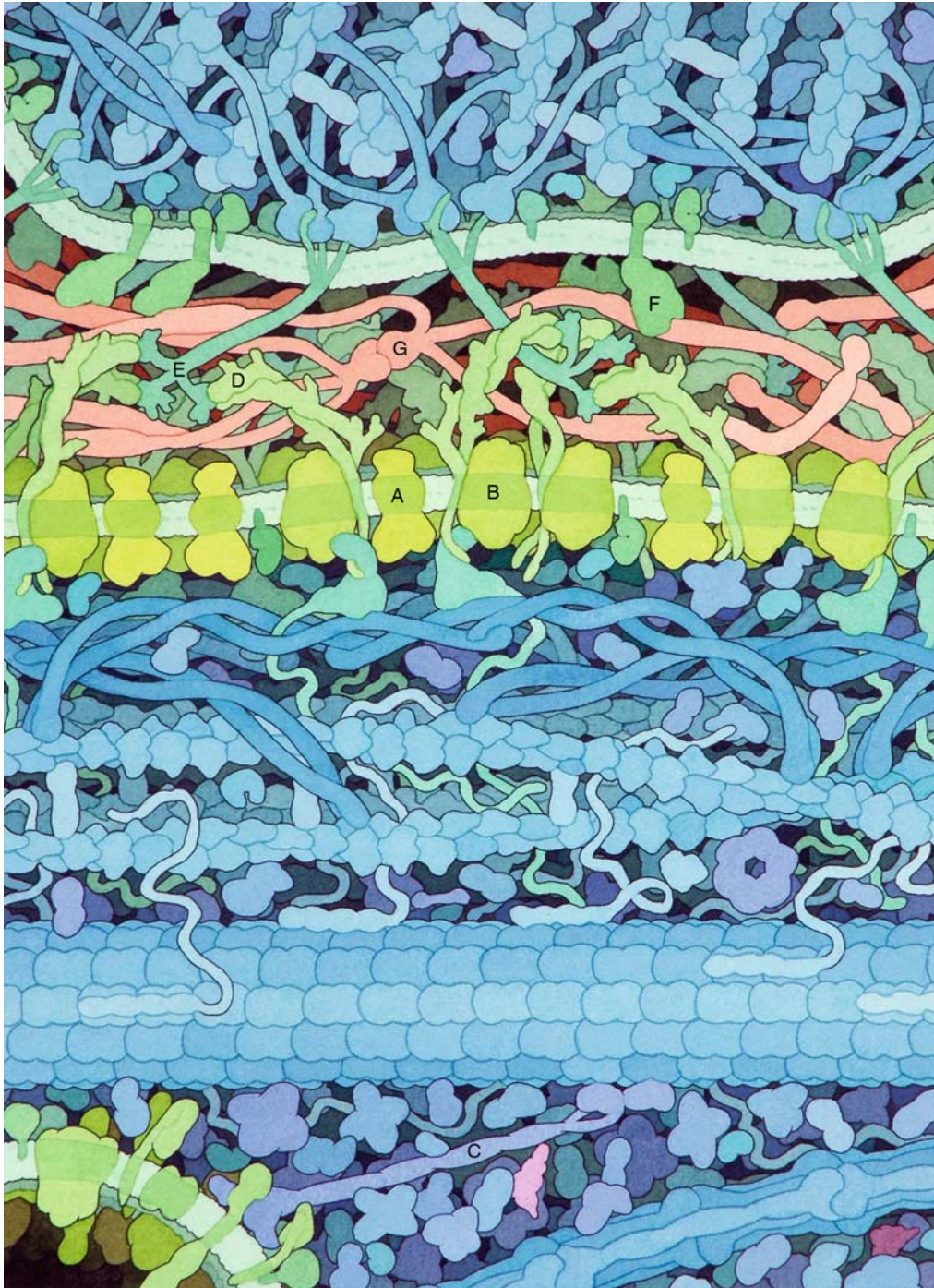
Nerves

Nerve cells specialize in rapid communication over long distances. We are wired with a network of nerve cells, all centrally controlled by the brain and reaching out to all parts of the body. Nerve signals shoot throughout the body, collecting and processing our senses and moving our muscles. Some of the signals never leave the brain, carrying our thoughts and storing our memories. By using different arrangements of nerve cells, and by varying the interaction between successive nerve cells, nerve networks can control the simplest hard-wired reflex responses or the most subtle thought processes.

Our nerves communicate with electrical signals and with chemical signals. The electrical signals are used to carry messages in a fraction of a second over long distances. The signals flash down the long, thin axons of nerve cells (Figs. 6.8 and 6.9), which range in length from very short axons in the densely packed neurons of the brain to over a meter long for the neurons that extend out to the distant extremities. Then, when the signal reaches the end of the axon, the cell releases neurotransmitters that carry a chemical signal across a narrow synapse to the next nerve cell.

The electrical signals are carried by chain of molecular relays that are powered by an electrochemical gradient of sodium ions. The process starts by charging the axon, making it ready to fire a signal. Pumps in the axon membrane transport sodium ions out of the axon into the narrow surrounding space, charging the membrane just like you would charge a battery. Then, a series of voltage-gated channel proteins use the charged membrane to propagate a message down the axon. The voltage-gated channel has an interesting property: if the membrane is charged, it stays closed, but if the electrochemical gradient drops (also causing the voltage across the membrane to drop with it), the channel opens up. This property allows it to propagate the nerve message.

The nerve initiates the signal by letting the sodium back across the membrane at the beginning of the axon. This reduces the voltage difference across the membrane. The channels in the local area sense this difference and all open up, letting even more sodium flow back into the axon. This triggers channels further down the axon to open, letting their local charge of sodium enter the axon. The signal flashes down the axon in a wave, as channels open down the entire length, leaving the entire axon uncharged after it is finished. The voltage-gated channels then spontaneously close and the chemical pumps get to work



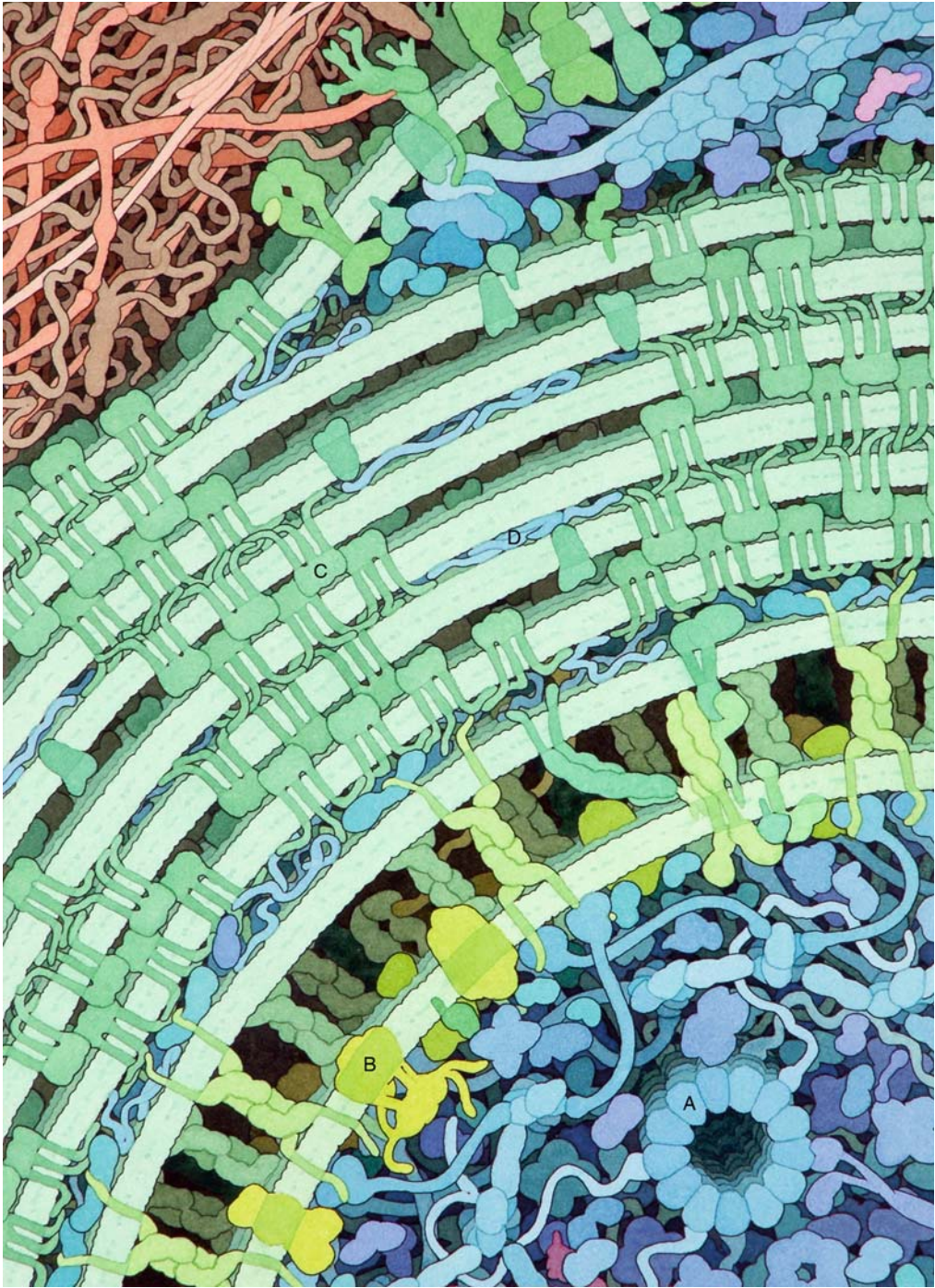
pumping sodium back across the membrane, preparing the axon for the next signal. It may sound complicated, but the whole cycle happens very fast. A typical nerve can fire off a signal over 200 times a second.

When the electrical wave reaches the end of the axon, it triggers the delivery of a chemical signal to the next nerve cell (Fig. 6.10). The drop in voltage causes the axon terminal to dump the contents of small vesicles into the narrow synaptic cleft between the cells. In some very active synapses, like the ones between nerves and muscle cells, several hundred vesicles are emptied, sending a strong signal. Other synapses, such as some of the connections in the central nervous system, send weaker messages that involve only a single vesicle. Each vesicle contains several thousand neurotransmitter molecules, which diffuse quickly across the synapse and bind to specific receptor proteins on the neighboring nerve cell, causing them to open. This allows thousands of ions to rush into that cell, and the signal begins once again.

Nerve cells typically have thousands of axon terminals ending on their surface, so they receive messages from many different cells. By using chemical signals, they can distinguish one message from the other, and decide what to do based on the results. Some synapses use neurotransmitters that deliver a positive, excitatory message, eliciting an electrical signal in the receiving cell. Other synapses use different neurotransmitters to deliver an inhibitory signal that suppresses an electrical response. Each nerve cell may receive thousands of these chemical signals, both excitatory and inhibitory. The relative number and timing determine whether an electrical signal is sent down its own axon. In this way, each nerve cell becomes a processor of information instead of a passive relay of a single message.



Fig. 6.8 Nerve Axon A cross-section through a nerve axon is shown here. The excitable cell membrane that carries the electrical nerve signal runs horizontally through the center of the illustration. This membrane is filled with two types of proteins, the sodium pump (A) that charges the membrane and the voltage-gated sodium channel (B) that propagates the electric signal down the membrane. A complex infrastructure of cytoskeletal proteins, shown here below the membrane, supports the axon and provides a railway for transport of resources up and down the axon. At the very bottom, kinesin (C) is shown dragging a small vesicle along a microtubule. A neighboring glial cell is shown at the top. The two cells are glued together by proteins such as neurofascin (D), gliomedin (E), integrin (F), and tenascin (G) (1,000,000 X)

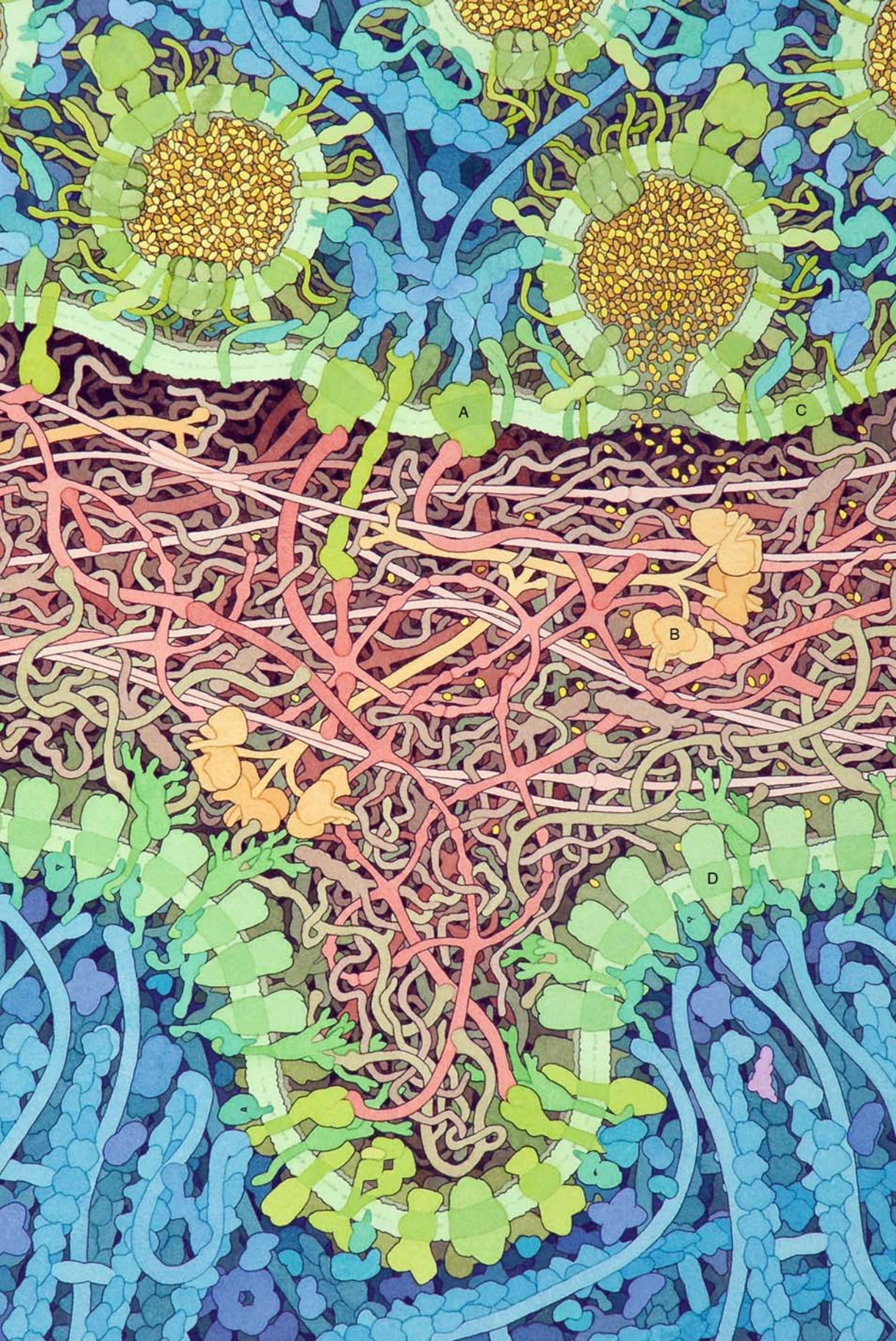


Of course, nerve signals flashing around the body are only useful if they do something. The nervous system is connected to the real world through a series of inputs and outputs. Sensory information feeds into the nervous system, keeping us up-to-date on the state of our surrounding environment. Each of the senses has a different molecular machine that probes an environmental variable. The eyes have light-sensitive rhodopsin proteins that initiate nerve signals when they absorb photons. Taste buds in our tongue have receptors that sense the level of ions or acid, and send signals that are interpreted as “salty” or “sour.” Receptor proteins in our ears sense the motion of small, hair-like protrusions on cells, interpreting these motions as the different pitches of sound that we hear. Ultimately, these sensory messages are all converted into electrical impulses that travel down nerve axons to the brain.

Nerves make their signals felt throughout the body by stimulating muscles and glands. We have conscious control of some of this: by thinking about it, we can send nervous signals down to the muscles in our arms and legs and cause them to contract. But much of the output of the nervous system is done without our conscious control. Nerve signals are sent to our internal muscles, guiding the beat of our heart and the slow motions of digestion. Nerve signals stimulate our glands to release the proper hormones, guiding our daily cycles and changes of life.



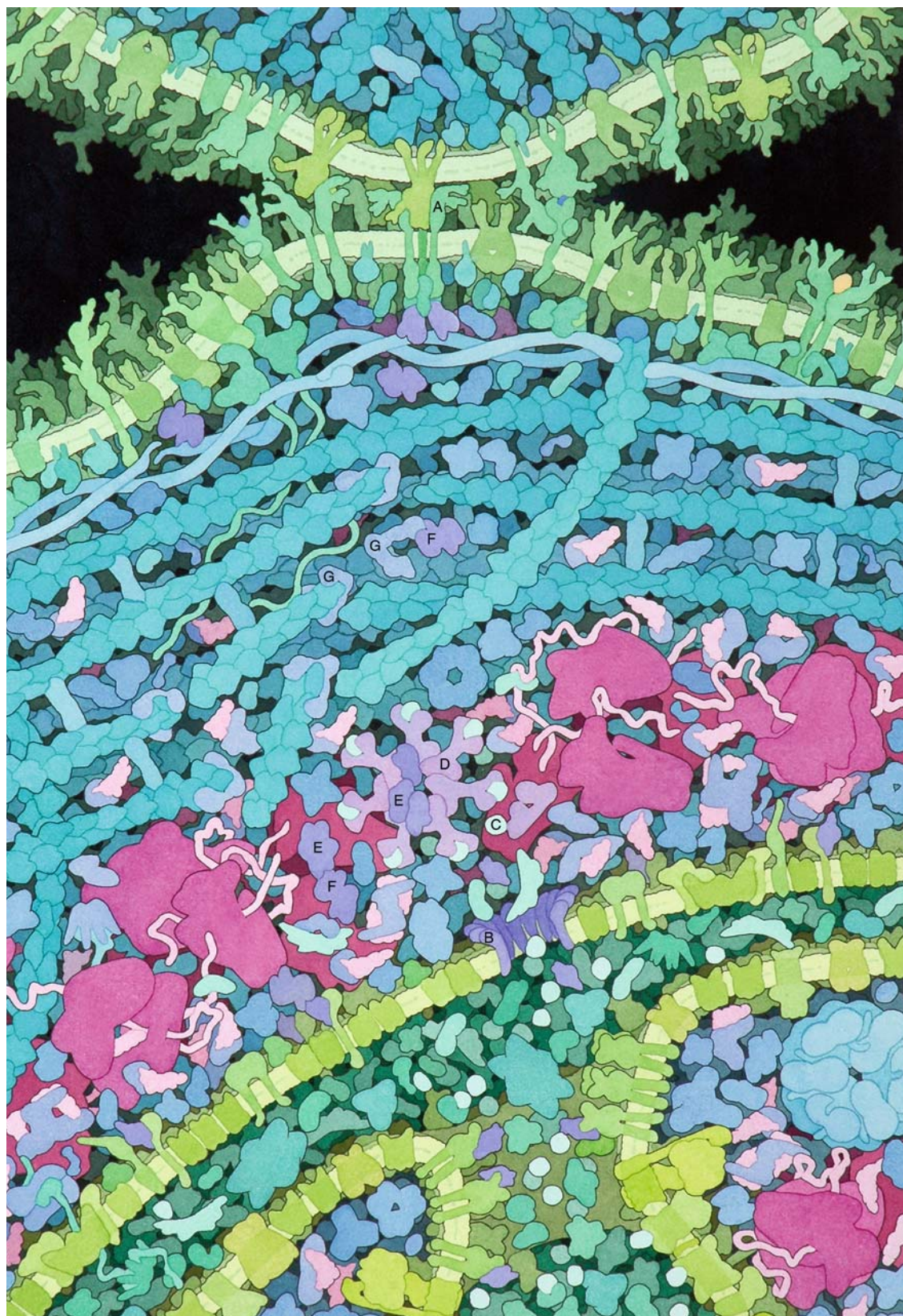
Fig. 6.9 Myelin Sheath The speed of transmission in our nerves is greatly increased by the use of a myelin sheath. A neighboring cell wraps layer after layer of insulating membrane around the axon. The length of the axon is composed of long segments of myelinated axon separating small segments of excitable membrane like that shown in Fig. 6.8. The nerve signal jumps quickly between these little segments of excitable membrane through the myelinated segments. Without the myelin sheath, a typical nerve can send signals at a rate of about 5 m/s, but it can be 10 times faster when the signal takes these shortcuts through the myelinated segments. In this cross-section, the axon is at the lower right, cut at an angle perpendicular to the previous illustration. One of the microtubules inside the axon is sliced at the very bottom (A). Myelinated axon membranes have far fewer pumps and channels than the unmyelinated segments, including the voltage-gated potassium channel shown near the bottom (B). At the top, a Schwann cell has wrapped four times around the axon, producing eight layers of insulating membrane. Proteins such as protein zero (C) and myelin basic protein (D) help the Schwann cell spread itself thin enough to form these densely stacked layers (1,000,000 X)



The hundred trillion cells in our brain control everything, processing inputs from all corners of the body and making the proper outputs. This amazing network was wired in our first few months of life. As our brain developed as an embryo, the nerve cells multiplied and extended many connections to their neighbors, wiring the different portions of the brain involved in sense, motion, and thought. Then, as we grew and learned, our many childhood experiences strengthened connections, remodeling the brain into an efficient machine for biological computation. Together, these cells guide the cycles of sleep and wakefulness, they interpret signals as pleasurable or painful, they recognize colors and sounds and words, they remember things that we have done in the past, they figure out what to do in tricky situations in the present, and they plan where we are going in the future.



Fig. 6.10 Nerve Synapse Chemical signals are transmitted from one nerve cell to the next across synapses, shown here in cross-section. The end of the axon is shown at the top, with two vesicles full of neurotransmitters inside the axon terminal. The vesicle on the left is docking to the membrane and the one on the right has fused with the membrane and is releasing its neurotransmitters. A complex collection of tethering and regulatory proteins in the vesicle membrane are needed to perform this delicate task, and voltage-gated calcium channels (A) in the nerve membrane help decide when to begin. The synapse between the cells is filled with basement membrane, which also includes the enzyme acetylcholinesterase (B) that breaks down the neurotransmitter after it is finished with its job. The small protein CHT1 (C) transports the pieces back into the cell to be recycled for the next nerve impulse. A muscle cell is shown at the bottom, with many acetylcholine receptors (D) on its surface. A tangled network of proteins inside the muscle cell holds the receptors in place, clustering them within the synapse (1,000,000 X)



Chapter 7

Life and Death

Decay and death are an inevitable consequence of the world that we live in. The forces of entropy wage a slow but implacable battle of erosion on everything. The major discovery made by the earliest cells, which allows life in our world of entropy, is the ability to maintain order when challenged by this inevitable decay into equilibrium. We might imagine two ways to face this great challenge: immortality or planned obsolescence. An immortal organism would be completely resistant to environmental forces, or have powerful repair mechanisms to undo damage as it occurs. This obviously was too much to ask for the earliest cells, which were built from fragile organic materials. Instead, they developed along a path of planned obsolescence (Fig. 7.1). Molecules, cells, and organisms are all born perfect and new, they live for a minute, a year, or a century, and then they die. . .but not before reproducing to create a new molecule, cell, or organism.



Fig. 7.1 Programmed Cell Death All of our cells are preprogrammed with a method to die on command, if necessary. When a cell enters into programmed cell death, it disassembles its own molecular machinery in a safe and orderly manner. In this illustration, a cytotoxic T-cell at the top has given the signal for the cell at the bottom to die. Proteins on the T-cell surface are recognized by death receptors (A) on the cell surface, beginning the process that leads to death. As part of the process, the BID protein (B) forms a pore in the surface of mitochondria (shown at the bottom), releasing cytochrome c (C) into the cytoplasm. This is the signal to assemble an apoptosome (D), which then activates initiator caspases (E). These then activate more caspases (F), launching an orderly campaign to attack key proteins throughout the cell. For instance, caspases make a cut in the protein gelsolin (G), converting it into an active form that disassembles actin filaments (1,000,000 X)

Within this cycle of birth, life, and death, there is one strand of immortality that lives through the centuries: the genetic information passed from generation to generation. But even this is changeable, since it gradually mutates and shifts, giving rise to the diversity of life that we now enjoy.

Ubiquitin and the Proteasome

Proteins are built for a given task, and then they are quickly discarded when the task is finished. In a typical cell, 20–40% of newly synthesized proteins will be destroyed within an hour, and some proteins with time-critical functions, such as regulators of transcription or cell division, only last for minutes. This approach of planned obsolescence may seem wasteful, but it has a great advantage. It allows the cell to respond quickly to changes in the environment.

Of course, this poses a challenge to the cell. You can't simply build protein-destroying enzymes and release them indiscriminately into the cytoplasm. Like the digestive enzymes in our stomach, they would destroy everything in sight. Instead, the cell builds proteasomes (Fig. 7.2). Proteasomes are voracious protein shredders, but the protein-cutting machinery is carefully hidden away inside a barrel-shaped structure. So, the proteasome is able to wander freely inside the cell, and only the proper proteins are fed into its hungry maw.

Cells need to have a way to control the destruction of proteins, making sure that only obsolete or damaged proteins are fed into the proteasome. The small protein ubiquitin plays a central role in this process. It is attached to old proteins, signaling to the cell that they are ready to be disassembled and recycled. The tricky part of this process is making sure that ubiquitin is only attached to the proper proteins. This is the job of a series of enzymes called ubiquitin ligases. With the help of two other enzymes, they recognize short-lived proteins and attach ubiquitin to them. A string of four or more ubiquitin molecules targets a protein for destruction. Caps on either end of the proteasome recognize this string of ubiquitin and feed the attached protein into the destruction chamber, where it is clipped into small pieces, ready for recycling.

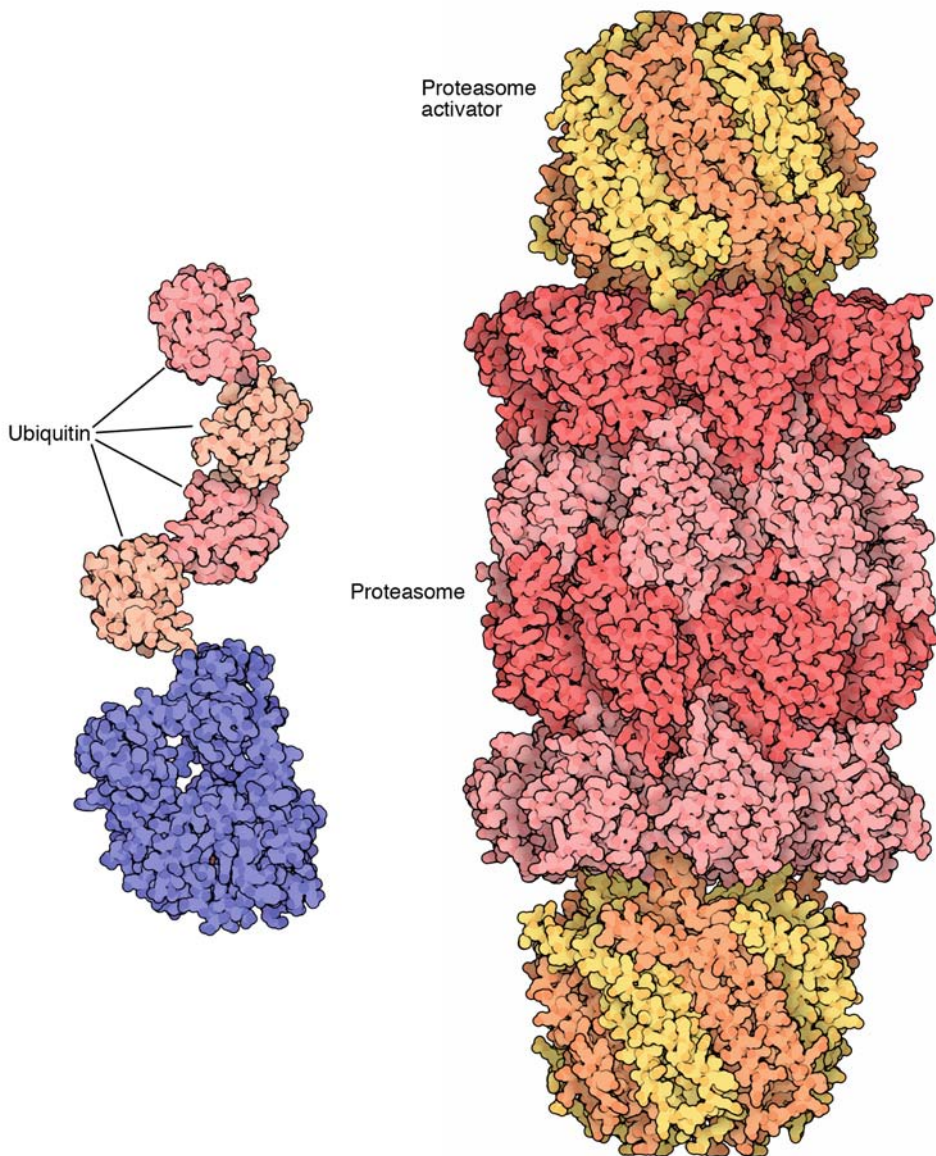


Fig. 7.2 Ubiquitin and the Proteasome When old proteins are ready for recycling, the cell attaches strings of ubiquitin to them. The proteasome then recognizes this ubiquitin and destroys the attached protein. The proteasome is composed of four stacked rings of protein, with the protein-cutting machinery hidden inside. The structure shown here also has a ring of activating proteins at either end. They selectively feed small peptides into the proteasome for complete destructions. A larger cap (not shown) is added at each end to recognize ubiquitin and feed in entire proteins (5,000,000 X)

DNA Repair

Our molecular machines are constantly under assault by environmental attackers. Reactive chemicals corrupt them, heat unfolds and denatures them, and ultraviolet light breaks them. In many cases, these molecules are ruined and can no longer perform their functions. The cell can afford to discard damaged proteins, but that is not the case with DNA. It must be kept in perfect form, since it carries the vital genetic information that guides the life of the cell and must be passed on to offspring. To ensure that this information is not lost, the cell has many different methods to protect the DNA from damage, and repair damage if it occurs.

Sunlight is a major danger to DNA. The ultraviolet light in sunlight is energetic enough to attack the nucleotides in DNA. The most dangerous wavelengths of UV light (termed UVC) are screened out by the ozone in the upper atmosphere, but the weaker rays, termed UVA and UVB, pass through the atmosphere and are powerful enough to cause chemical changes in DNA. The ultraviolet light is absorbed by a double bond in thymine and cytosine nucleotides, causing them to react with neighboring nucleotides (Fig. 7.3). If the neighbor is another thymine or cytosine, it can form a bond between the two nucleotides, gluing them together. This unnatural linkage forms an awkward kink in the DNA and causes problems when DNA polymerase tries to replicate the DNA strand. In particular, a connection between two neighboring cytosines will often lead to a mutation, since DNA polymerase may wrongly pair adenine with these corrupted bases instead of the proper guanine. As described below, this can lead to major problems like cancer.

Damage by ultraviolet light is not a rare event. Every second you are in the sun, 50 to 100 of these unnatural bonds are formed in each skin cell! So it will come as no surprise that cells have efficient methods for correcting these mistakes. Our cells use a process called nucleotide excision repair, which requires the concerted effort of many enzymes. Some recognize the stiff kink formed by the troublesome bond, others clip out the section of DNA, and the rest build a new copy of the damaged area. The entire process relies on the fact that the complementary strand in the DNA helix is still undamaged, and can be used to build a replacement for the damaged strand. Other organisms take a more direct approach. They have enzymes that seek out the two corrupted bases and correct them directly. For instance, the photolyase shown in Fig. 7.3 binds to the damaged DNA and breaks the

bond between the two bases. Ironically, the reaction performed by this enzyme is powered by visible light.

Long, delicate DNA strands are also easily broken. X-rays and gamma rays, as well as the enzymes of respiration, create dangerously

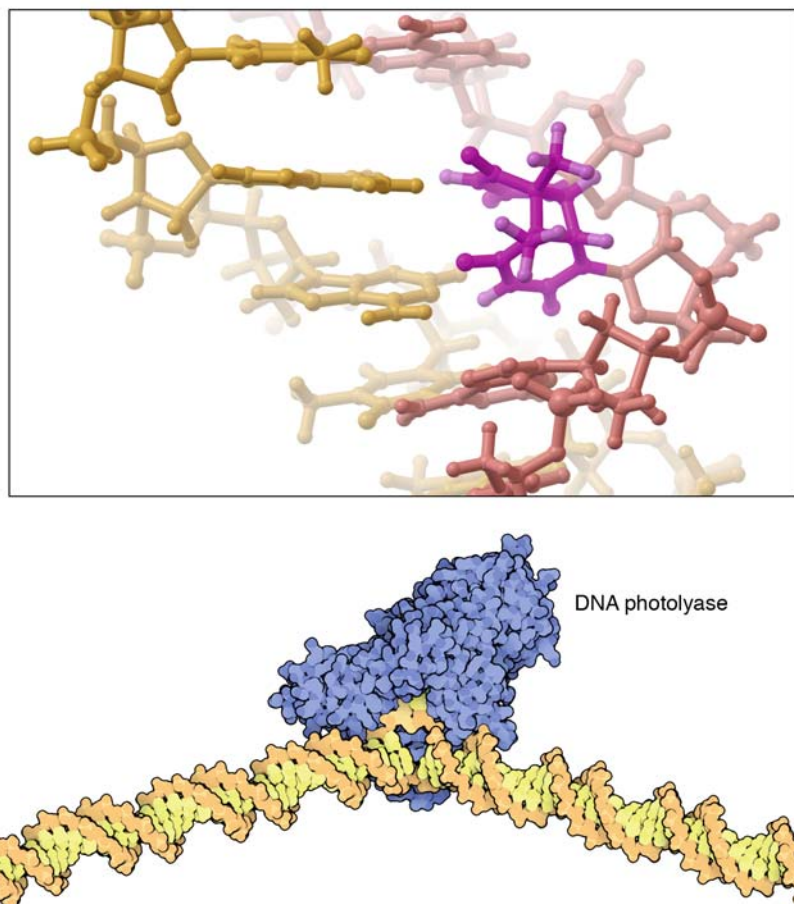


Fig. 7.3 DNA Photolyase DNA photolyase binds to DNA with damaged nucleotides, and breaks the unnatural bond between them. It is not gentle with the DNA—it wrenches the damaged bases out of the normal stack and performs the reaction in a small pocket. The upper diagram shows a close-up of the damaged bases (in this ball-and-stick diagram, the bonds connecting atoms are shown as cylinders). Notice how the two unnaturally bonded bases, shown in magenta, are distorted out of the normal stacking pattern. The lower pictures show the enzyme correcting the problem (top, 40,000,000 X; bottom, 5,000,000 X)

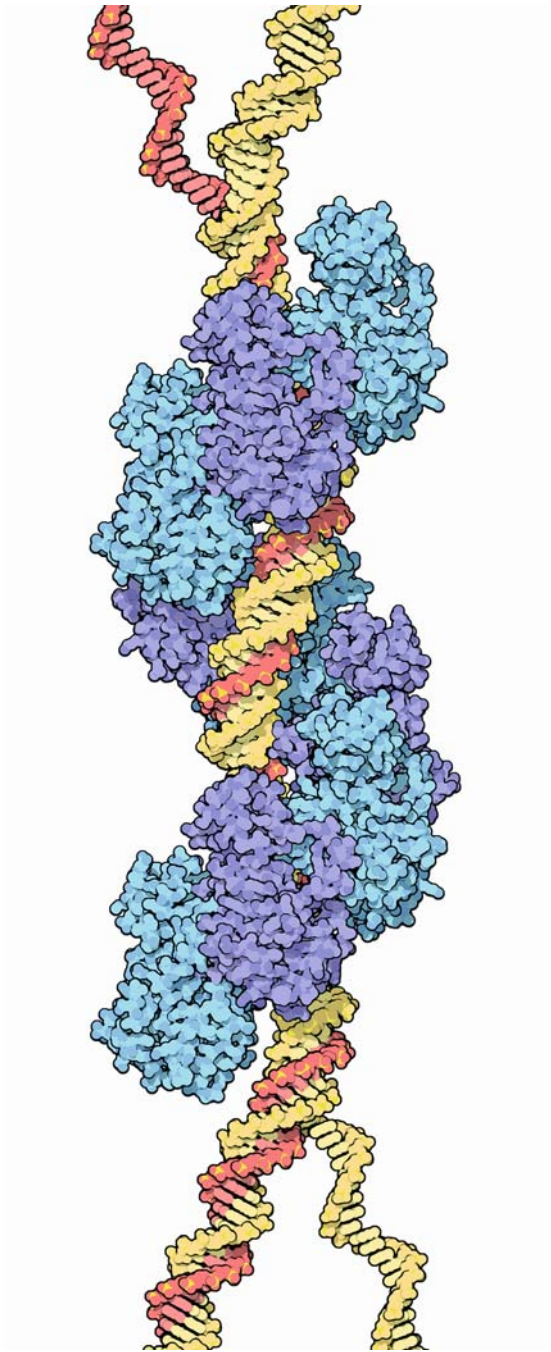
reactive forms of oxygen (described in more detail below) that cause breaks in the DNA. Breaks can also occur when polymerases and topoisomerases make errors. In addition, cells occasionally break their own DNA on purpose, for instance during the shuffling of genes that is used to create different antibodies. Occasionally, they make a mistake when reconnecting the strands. A break in one strand of an important gene can kill the cell, so cells have methods for repairing breaks and keeping the genome intact.

Homologous recombination is the primary method for repairing breaks in the DNA. It relies on the fact that each cell carries a duplicate set of DNA. The break is repaired by using the duplicate set as a template to match and connect the broken strands. The central step of the process is called synapsis, where the two homologous strands—the damaged one and the undamaged template—are brought together in perfect alignment. The intact copy of the DNA is unwound and the damaged strand is paired with it. Then, this paired form is used to reconstruct the missing pieces and reconnect the strands. This amazing process is performed in our cells by the protein Rad51 (Fig. 7.4), and by the similar protein RecA in bacterial cells (shown in Fig. 4.7). These proteins form long helical filaments that surround the DNA strands, holding them in close proximity and allowing a perfect match to be found.

Cells also have a less accurate method for repairing breaks that doesn't need a template strand. It is termed non-homologous end joining, and it repairs the break directly without the need for outside information. The process starts when two proteins bind to the ends of the broken strands, and bring them together. Then, special nucleases and polymerases trim the ends and fill in any gaps, making them ready for rejoining. Finally, a DNA ligase glues the ends back together. In the process of trimming the ends, a few nucleotides may be lost, so this repair method can lead to potentially dangerous changes in the genetic information. But it is better than a broken DNA, which would be lethal to the cell.

Telomeres

The ends of DNA strands are susceptible to several serious problems. First, they are subject to unwinding, so they are sensitive to degradation. Also, DNA polymerase has trouble copying DNA all the way to the end, so after several rounds of replication, the DNA gets gradually



shorter and shorter. Many bacteria combat this problem by getting rid of the ends entirely: they close their DNA into a big circle. Our cells, however, contain 46 linear strands, each with two ends that must be protected.

To solve this problem, our DNA strands have a special nucleotide sequence at each end, called a telomere. The telomere is composed of the sequence GGGTTA repeated over and over, about a thousand times in a row. A set of special proteins binds to the telomere, wrapping it into a loop, thus sealing off the end and protecting it from DNA-cutting enzymes. The telomere also solves the problem of shortening. When the cell divides and the DNA is replicated, the telomere loses 50–100 bases off of each end. Then, the enzyme telomerase binds to the telomere, and using its own internal RNA template, it extends the telomere with new copies of the repeated sequence. Since it is a repeated sequence, it doesn't really matter how much it adds, as long as it adds enough to keep up with the losses.

Embryonic cells and stem cells, like the ones that continually produce blood cells throughout our lives, have an active telomerase that protects their DNA during replication. However, most of our cells have turned off their ability to extend telomeres. For instance, adult human fibroblasts can divide about 60 times before irreparable damage occurs and the cells die. This may serve to protect us from cancer by providing a safety check on the growth of cells. If suddenly a group of cells starts growing without control, they will all die off in a few dozen generations as their telomeres shrink to nothing.

Programmed Cell Death

Cells that are gravely injured make a mess when they die. They swell and burst, and the contents of the cell spill out and get into inconvenient places. Lysosomes (small compartments that perform digestion inside cells) may be damaged, releasing their destructive enzymes.

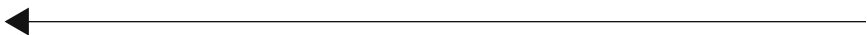


Fig. 7.4 DNA Strand Exchange Rad51 proteins form a helical filament that encloses strands of DNA. In this illustration, a single strand of DNA (in red) is being paired with a double helix (in yellow) that has the same sequence of nucleotides. The strands enter at the top, exchange strands in a hybrid triple helix structure, and exit in a new pairing at the bottom (5,000,000 X)

The body responds by inflaming the area with immune cells that struggle to clean up the mess without damaging the surrounding healthy tissue too much.

To avoid this messy problem, our cells are programmed with a method to commit suicide, quickly and cleanly. This process, called programmed cell death or apoptosis (Fig. 7.1), allows the cell to disassemble itself in an orderly fashion and notify the immune system that it is ready to be recycled. Cells trigger apoptosis for many different reasons. If they are damaged—for instance, if the DNA is broken in many places or if the cell is infected with a virus—they may enter programmed cell death. It is also important in development. For instance, our toes were created when we were still embryos with the help of programmed cell death. A flat flipper-like appendage was created first, then small patches of cells died cleanly to separate the toes. A similar process occurs with tadpole tails as they mature into frogs. Programmed cell death also plays an important role in protecting us from cancer, since cells that show abnormal growth are usually forced to die.

Of course, there must be checks and balances on this deadly system. Cells must ensure that apoptosis is triggered only when absolutely necessary. Apoptosis is controlled by a tribunal of proteins, known collectively as the Bcl-2 family of proteins. Together, they weigh the pros and cons of cell death at any time. Some of these proteins are pro-survival. When the cell is healthy and useful, these proteins dominate and they suppress the signals that lead to apoptosis. But if DNA damage or infection is sensed, or if cells become detached from their neighbors, a second set of proteins sentence the cell to death.

Caspases are the ultimate executioners of programmed cell death (Fig. 7.5). They are protein-cutting enzymes similar to the enzymes that digest our food. They are far more selective than our digestive enzymes, however, and the targets of the caspases are carefully chosen to allow the piecewise destruction of the cell. The division of the cell is halted by the destruction of key regulatory proteins, and polymerases are destroyed, halting the synthesis of new nucleic acids. Structural proteins, such as the lamins that support the membrane around the nucleus, are cleaved and disassembled. To facilitate rapid processing, adhesion proteins on the cell surface are cleaved, releasing the cell from its neighbors. Finally, the cell membrane is subtly changed, signaling to the surrounding tissue that the cell is ready to be absorbed and recycled.

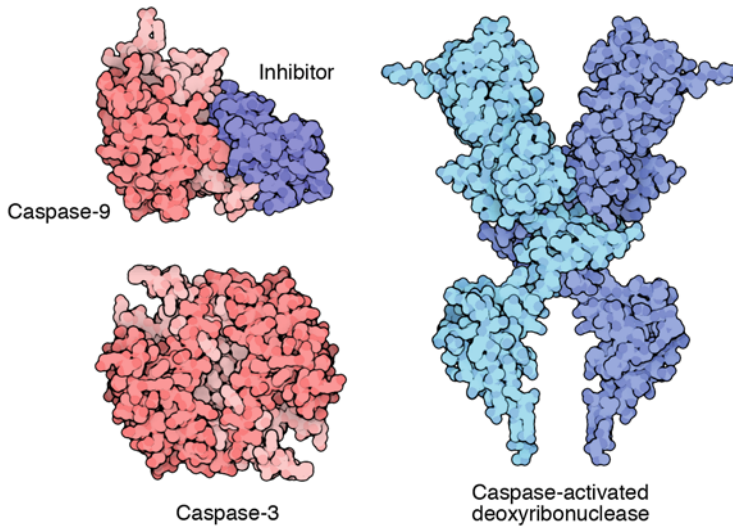


Fig. 7.5 Caspases Caspases methodically disassemble proteins during programmed cell death. Caspase-9 is an initiator caspase that is normally held in an inactive state by a small inhibitor protein, shown in blue. When programmed cell death is initiated, this inhibitor is removed and the caspase makes a small break in several effector caspases, such as caspase-3. The effector caspases then attack proteins throughout the cell. They also activate other destructive machinery, such as this deoxyribonuclease. It grabs DNA strands in the large groove at the top and clips them into small pieces (5,000,000 X)

Cancer

Growth of new cells is a highly regulated event that requires the permission and cooperation of entire tissues. Cells are constantly communicating with one another, deciding moment-by-moment if new cells are needed. Tissues like the skin, the blood, and the digestive system need new cells continually, to replace cells that are lost or worn out. Other tissues, like the brain, do not require this much replacement and the cells divide more rarely. Normally, through careful communication and control, our cells divide just enough to keep their local tissues healthy and active. However, when this communication fails, abnormal cells may grow without control, leading to cancer.

Our bodies have many protections against cancer, so cancer cells must make many changes to be dangerous. They need to be able to ignore the many signals from their neighbors, as well as their own

internal signals, that are all telling them not to grow. They need to be able to convince their neighbors to build new blood vessels to supply the abnormally growing tumor with extra food and oxygen. With particularly invasive cancers, they have to corrupt the normal molecular machinery for crawling and digesting, and use it to force their way into surrounding tissues, and through the bloodstream, into distant parts of the body.

All of these changes are made by mutating specific proteins in the cancer cell, or building too much or too little of a key protein. The genes encoding these proteins are often termed oncogenes because of this connection to cancer. For instance, one of the central oncogenes in many cancer cells is a mutation in the p53 tumor suppressor protein. It normally watches the cell for damage to the DNA and other changes that might lead to aberrant growth. If it finds damage, it can freeze the division of cells or even cause programmed cell death. Many cancer cells, however, have a mutated form of p53 tumor suppressor that is no longer effective, so the cancer is free to grow without control. Other oncogenes that may be mutated in cancer cells include signaling proteins that communicate with neighboring cells (Fig. 7.6), adhesion proteins on the cell surface, and proteases that cut the connective tissue between cells.

We start out life with a clean set of genes, all of which support the normal growth and maintenance of tissues. As we grow older, however, our genes are continually attacked by sunlight and chemicals, causing mutations at random places in the DNA. As described above, many of these mutations are corrected by our repair mechanisms, but some slip through. Many are harmless, but as we grow older and older, more and more of them build up. If just the right combination of mutations occurs in a single cell, it becomes a cancer cell and grows into a tumor.

Cancer is particularly difficult to treat, since cancer cells are basically human cells that have gone bad. Surgical and radiation treatments are the most direct: they simply cut or burn away the damaged cells. Chemotherapy works a different way. Anticancer drugs attempt to take advantage of the differences between cancer cells and normal cells. Unfortunately, these differences are subtle, and most of the anticancer drugs in current use take advantage of the main property of cancer cells: that they grow fast. These drugs attack different aspects of cell division, blocking the replication of DNA or the machinery that separates two daughter cells. This effectively poisons the cancer cells, but it also kills any normal cells that are rapidly growing, such as the cells in hair follicles and the cells that line and protect the digestive system. This leads to the severe side effects of chemotherapy.

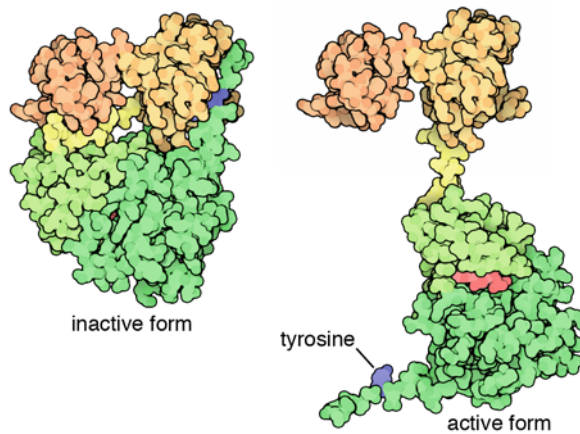


Fig. 7.6 The src Oncogene Src protein relays messages from receptors on the cell surface, stimulating proteins that control cellular structure, cell communication, and cell growth. It is normally bundled up tightly, as shown on the left, in an inactive form. But when it gets a message from the cell surface, a phosphate is removed from a special tyrosine amino acid (colored blue here). This allows the protein to open up and become active as an enzyme. It then hops from protein to protein, spreading the signal by adding phosphates to them, using ATP (red) as the source of the phosphate. When the signal is done, it folds back up and waits for the next message. In cancer cells, however, the src gene is often mutated. Either the special tyrosine is mutated or the entire tail with the tyrosine is deleted, so the Src protein cannot close up. The mutated Src protein remains active all the time, stimulating uncontrolled growth (5,000,000 X)

Aging

When we are born, our cells are brand new and functioning according to the genomic plan. As we grow older, however, our molecules and cells and bodies slowly age, becoming less efficient and ultimately leading to failure and death. As you might imagine, aging has been a subject of intense medical research, as we search for ways to slow the aging process. In spite of this work, it is still a mysterious topic with many unknown quantities. However, a few key findings have revealed some of the major factors. Looking at a variety of different animals, we find that the maximal lifespan is tied to body size and to the rate of metabolism. Small animals have very fast metabolism, so they age quickly and die younger than larger animals. Research has shown that a major underlying cause of this aging is the slow but steady accumulation of damage from reactive forms of oxygen.

Our cells rely on oxygen as the final acceptor of electrons in respiration, allowing us to extract far more energy from food than would be possible without oxygen. However, dangerously reactive forms of oxygen, such as superoxide and hydroxyl radicals, are formed during these reactions. Occasionally, these toxic molecules leak away from the respiratory enzymes before they can be fully converted to water (Fig. 7.7). They then attack proteins and DNA, damaging them or causing mutations.

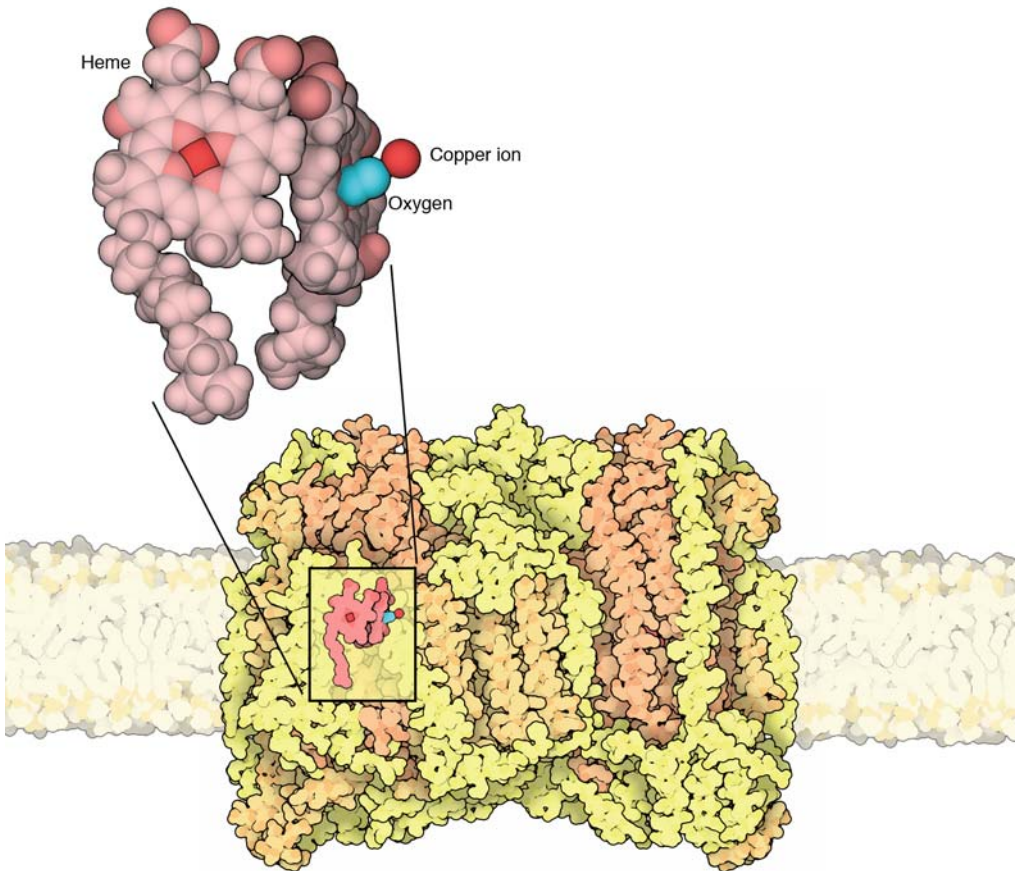


Fig. 7.7 Cytochrome c Oxidase Cytochrome c oxidase is the major culprit in the formation of reactive oxygen molecules. It performs the last step in respiration, placing the electrons extracted from food molecules onto oxygen. It is a large protein complex found in the mitochondria. The active site is buried deep inside the protein, and several heme molecules and copper atoms assist with the reaction. The oxygen molecule is shown in bright blue. (top, 20,000,000 X; bottom, 5,000,000 X)

They also attack lipids in our membranes, creating reactive forms that can then go on to attack other molecules. The liver spots that form on aging skin are a visible sign of this. They are composed of lipofuscin, a dark peroxidized form of our normal lipids.

Oxidation is not a minor problem. Our respiratory system is one of our most active molecular systems and these reactive forms of oxygen continually flood through the cell as a byproduct. Fortunately, we have an effective mechanism that protects against this ever-present danger. Our front line of defense is a series of enzymes that seek out reactive forms of oxygen and detoxify them. These enzymes include superoxide dismutase, which destroys superoxide (oxygen with an extra electron), and two enzymes that destroy peroxides (Fig. 7.8).

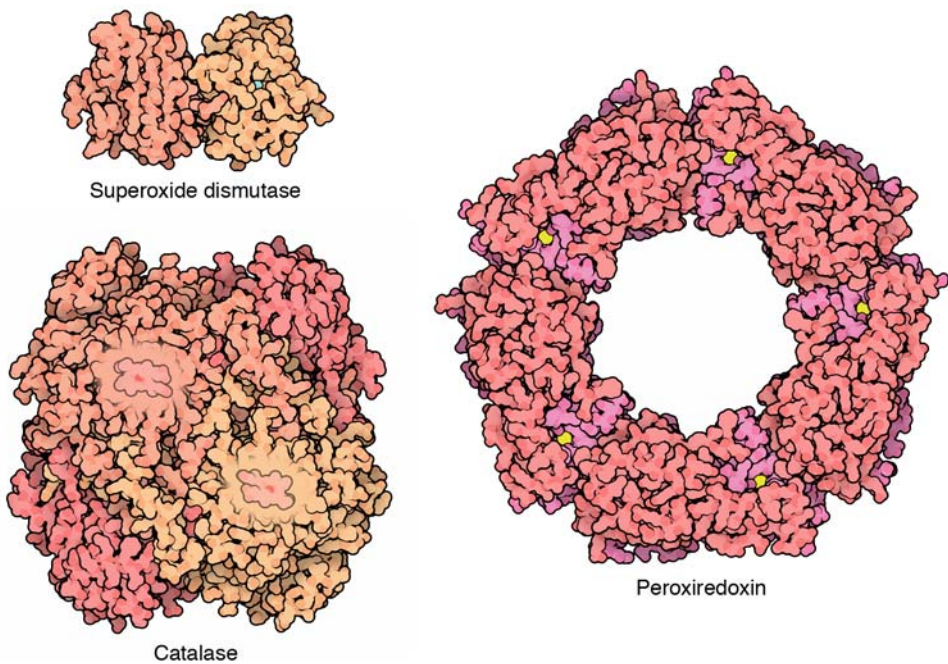


Fig. 7.8 Antioxidant Enzymes Our cells have several enzymes that detoxify reactive forms of oxygen. Superoxide dismutase detoxifies superoxide molecules, and catalase and peroxiredoxin destroy hydrogen peroxide. Each of these enzymes uses a special chemical tool in the reaction. Superoxide dismutase uses copper and zinc atoms (one is seen in the active site here, in bright blue), catalase uses iron ions trapped in heme molecules, and peroxiredoxin uses a particularly reactive sulfur atom in a cysteine amino acid (colored bright yellow here) (5,000,000 X)

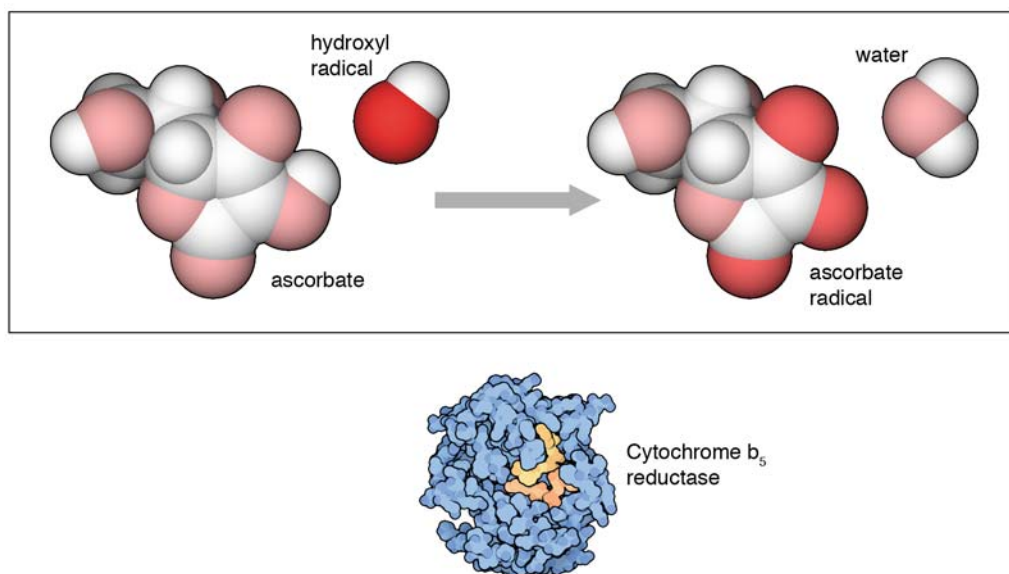


Fig. 7.9 Vitamin C Vitamin C battles free radical molecules one-on-one (free radicals are molecules that have a particularly reactive unpaired electron). At the top, vitamin C (ascorbate) donates a hydrogen atom to a hydroxyl radical. This leaves vitamin C as a free radical, but it is far more stable than the hydroxyl radical and does not attack other biological molecules as readily. The vitamin C radical is then detoxified by reductase enzymes, such as cytochrome b₅ reductase (bottom), adding the hydrogen back and making it ready to attack another hydroxyl radical. The protein is shown in blue, and two molecules that assist with the reaction are shown in orange and pink (top, 40,000,000 X; bottom, 5,000,000 X)

Several small antioxidant molecules also assist in this task. In the water-filled compartments of the cell, glutathione and vitamin C battle oxygen species one-on-one, detoxifying them if they find one (Fig. 7.9). Reactive forms of oxygen, however, are even more soluble inside membranes, so we have membrane-bound vitamin A and vitamin E to detoxify them. This function is so essential that our cells may have one molecule of vitamin E for every 100 lipid molecules!

You might think that it would be easy to slow the aging process by simply by flooding your body with more antioxidants, like vitamins A, C, and E. Many studies have been performed to test this idea, and the results have unfortunately been negative. In cases where a person has a naturally damaged system with sub-optimal levels of antioxidants,

supplements do show a significant effect, helping to reduce damage by reactive oxygen and slow aging. But for most of us, the normal level of antioxidants has been honed by evolution to provide us with the maximum possible protection, so supplements of additional antioxidants don't seem to help much more.

Another course of action, however, has shown an amazing ability to slow aging. Many animals show greatly increased lifespans, sometimes doubling the time they live, when they are given very limited diets. The diet still includes all of the nutrients needed for normal growth and maintenance, but the number of calories is greatly reduced so that the animal barely survives. The reason that the diet works is still a matter of controversy, but one theory links it back to damage by reactive oxygen. With the calorie-restricted diet, the metabolism is slowed, less oxygen is used and fewer molecules of reactive oxygen leak from the respiratory enzymes.

Death

Death is defined in the Random House dictionary as “the total and permanent cessation of all of the vital functions of an animal or plant.” However, in this era of advanced medical techniques, death is not such a well-defined concept. Victims of drowning or heart attacks who are seemingly dead may be revived by cardiopulmonary resuscitation, and temporary death-like comas may be induced with drugs to protect the brain during major surgery. We all know when an animal is alive: its heart beats, it breathes, it responds to pain and heat and cold. It's also easy to see when an animal is definitely dead and has begun to decompose. But defining the actual moment of death is still a mysterious gray area.

An exact definition is essential, however, in human affairs. There are legal and ethical implications. We need to know when a person has died so that we can proceed with the customary funeral rituals, and sort out the details of passing on their estate. In modern society, there is also the possibility of using organs for transplants, and this must be done very soon after death. In 1968, a committee at Harvard Medical School proposed a definition that is still used today to mark the exact time of death. They identify the nonreversible loss of all brain function as being the point of death. This is characterized by loss of all electrical function and blood circulation in the brain. The logic behind this cutoff is simple: it assumes that all of the important aspects of a

person—their thoughts, their memories, their personality, and their ability to control their body—is manifested in brain activity. When this is lost, the person has died.

After death, the body quickly succumbs to the forces of entropy. The body cools over a day or two as leftover metabolic processes run their course without the proper controls. Muscles seize up in rigor mortis and then relax as all of the calcium and ATP are used up and equalized throughout the cell. Then the body quickly degrades into decay as all forms of regulation and protection are lost. Most cultures have ways of disposing with human bodies before the corruption gets too severe, such as cremation, embalming, or burial.

Many parts of the body, however, last long after death. Bones and hair may last for years or centuries, though there is nothing living left in them. If they are fossilized, they can leave their traces for millennia. Researchers have taken advantage of this and begun looking for traces of biological molecules from organisms that died thousands of years ago. In some special cases, where the remains have been preserved particularly well, they are having success. For instance, short fragments of DNA have been recovered from the bones of a Neanderthal who lived 40,000 years ago, helping us fill out the complex genetics of our family tree. This type of DNA archeology can give us a glimpse of our distant ancestors, bringing them back to life in our mind's eye.



Chapter 8

Viruses

If you have ever had a cold, or chicken pox, or the mumps, or the flu, or measles, or any of dozens of other diseases, then you have been attacked by a virus. Viruses are an ever-present danger in our biological environment, and in spite of many powerful methods that protect us, sometimes they get the upper hand and pose a threat to life.

Viruses are completely selfish. They break into cells, overpower their normal functioning, and coerce them to one task: the production of more viruses. Most often, the cell is killed in the process. Looking at the three viruses presented in this chapter, we see that this is frighteningly easy to do. Viruses only need to do two things: they need to have a mechanism for creating new copies of themselves, and they need a way to get in and out of their target cells.

Viruses have a particularly simple way of creating new viruses, a way that requires only a minimal investment of molecular machinery. All they need to do is get a copy of a viral messenger RNA into a cell. This messenger RNA encodes all of the proteins needed to manufacture and assemble the component parts of the virus. In general, this only needs to include a few types of proteins: several proteins that

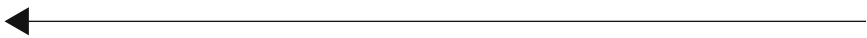


Fig. 8.1 HIV Human immunodeficiency virus is composed of a handful of different molecules that together are enough to infect and kill a human cell. The virus is surrounded by a lipid membrane stolen from an infected cell. The membrane is studded with GP120 (A), the protein that recognizes the next cell that the virus will infect, and supported on the inside by matrix proteins (B). Several enzymes float around inside, include HIV protease (C), reverse transcriptase (D), and integrase (E). The two RNA strands that carry the genome of HIV (F) are packaged in a cone-shaped container composed of capsid proteins (G) (1,000,000 X)

compose the shell that surrounds the mature virus, and a few proteins that build new copies of the RNA itself. Most viruses don't bother to encode any machinery to synthesize proteins from the viral RNA—they simply co-opt the ribosomes and transfer RNA molecules that are already there in the cell.

During the process of getting their viral RNA into the cell, viruses have no respect for the orderly flow of genetic information—from DNA to RNA to protein—that normally occurs in our cells. Poliovirus and rhinovirus take a shortcut and avoid DNA altogether. They inject a viral RNA into the cell, which contains instructions for making a special RNA-dependent RNA polymerase. This viral polymerase builds RNA strands using the viral RNA strand as the template. Thus, they don't need DNA at all—the viral RNA is copied directly to make more viral RNA. HIV (Fig. 8.1) uses an even more heretical approach. It also injects a viral RNA molecule, but then it uses a reverse transcriptase enzyme to make DNA based on the viral RNA template. The normal machinery of the infected cell is then forced to create RNA and protein from that newly minted viral DNA. Other viruses use all possible combinations, some injecting viral RNA and others injecting viral DNA, but all ultimately leading to a messenger RNA needed to build the viral proteins.

Targeting, entry, and exit are all performed by the package that surrounds a mature virus. This is where the wide diversity of viruses is seen. These range from tiny, perfectly symmetrical capsids like poliovirus to large membrane-enclosed structures like HIV and smallpox. By changing the proteins that are exposed on the surface, the virus can recognize different cells and infect only those that are most amenable to attack. Other proteins orchestrate the exit of viruses from the cell, either bursting the cell when it gets too full of new viruses, or budding one-by-one from the surface of the cell, slowly using up its resources as more and more viruses are made.

Many viruses also make a few additional proteins that dismantle the normal functioning of the cell. Some of these block the cell's protective mechanisms and others shut down synthesis of normal cellular protein, forcing the cell to make only viral proteins. For instance, our cells contain a radical defense against viruses. They contain a protein termed Pkr (protein kinase RNA-activated) that recognizes the unusual forms of RNA made by viruses and shuts down protein synthesis in an infected cell. Since all protein synthesis is stopped, the cell will die, but this will ensure that no viruses are made as well. Many viruses, however, fight back by creating a protein that specifically disables Pkr, keeping the protein synthesis machinery running and making more viruses.

Remarkably, these few ingredients—a tiny time bomb composed of RNA and protein, barely the size of a ribosome—are all that is needed to take over a cell. But without the molecular machinery of the cell, viruses are nothing. They cannot reproduce on their own. They cannot make any of their own amino acids and nucleotides and proteins. They are blind assailants, inert until they bump into the side of an unlucky cell.

Poliovirus and Rhinovirus

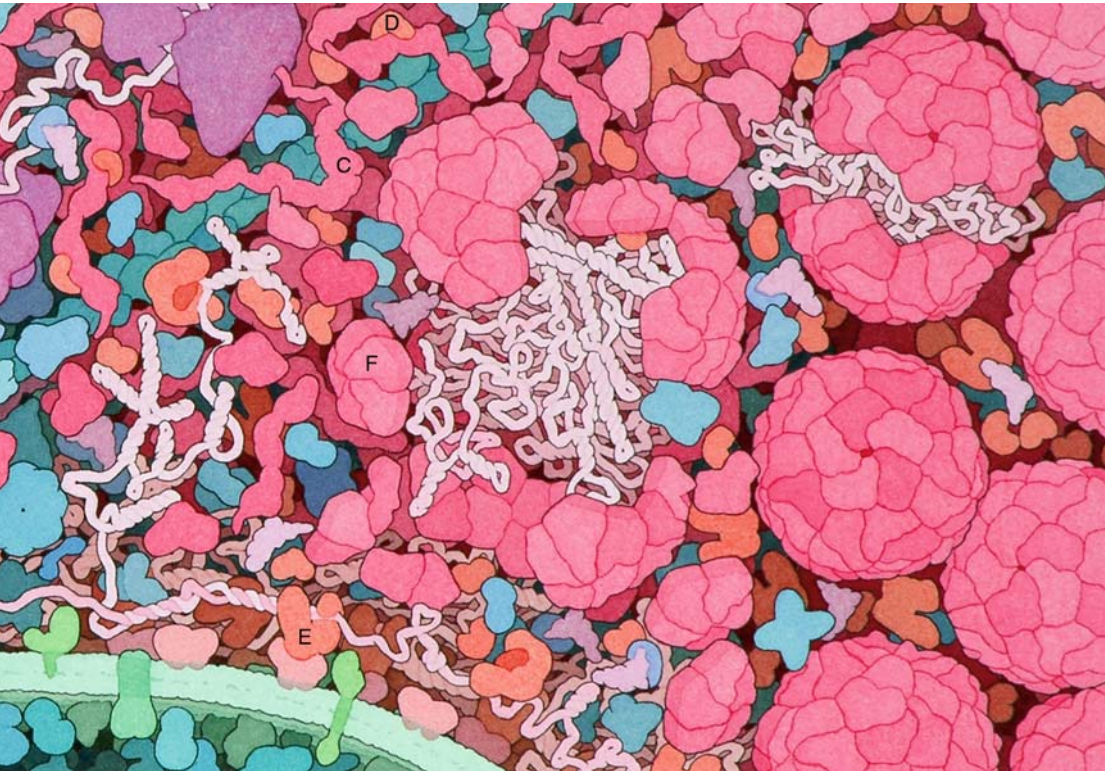
Poliovirus, the agent of poliomyelitis, and rhinovirus, one of several viruses that cause the common cold, are among the simplest viruses to attack human cells. They are composed of a single strand of RNA about 7500 nucleotides long, encapsulated in a symmetrical protein shell. But in spite of their simplicity, these viruses are remarkably fast and ruthless. For instance, in 4–6 h, a poliovirus can hijack a cell, shut down its normal protein synthesis, and force it to make 10,000 to 100,000 new viruses (Fig. 8.2).

A small difference in the protein coats of poliovirus and rhinovirus accounts for the widely different diseases caused by these viruses. When we swallow these viruses along with our food or when we breathe them in infected aerosols, the viruses infect different places. The protein capsid of poliovirus is perfectly stable in the acid environment of the stomach, so it infects there and can then spread through the lymph to distant parts of the body. Rhinovirus, on the other hand, is destroyed by acid and therefore infects primarily the throat and nose, which are not as acidic. The instability of rhinovirus keeps it confined to inconvenient places, so we get a runny, stuffed up nose as our cells attempt to protect themselves and flush out the attacking viruses. The stability of poliovirus, however, allows it to reach deeper into vital regions of the body. Most people infected by poliovirus experience only a bad fever, but in some infected people, the virus takes a stronger hold and causes serious damage to nerve cells and the brain.

The genomes of the poliovirus and rhinovirus are so small that only a few proteins may be encoded. These include four proteins that together make up the capsid, two proteases that cut the viral proteins into the proper lengths, a special RNA-dependent RNA polymerase that makes new RNA strands based on the viral RNA, and a few small proteins to assist the process. This handful of proteins, however, is all



Fig. 8.2 Poliovirus Life Cycle Poliovirus takes advantage of many of the molecular machines already available in cells during its life cycle. Viruses recognize and bind to glycoproteins on the cell surface (A) and force their viral RNA (B) inside. Then the cell's ribosomes build a long viral polypeptide (C) from the RNA. This is broken into several functional pieces by a viral protease (D), which amazingly first must cut itself out of the



polyprotein. One of these pieces is a special polymerase (E) that can build more RNA strands using the viral RNA as a template. Repeated rounds of synthesis build up large quantities of viral RNA and capsid proteins (F), which associate spontaneously into mature viruses and ultimately burst out of the cell (1,000,000 X)

that is needed to orchestrate a life cycle that ends in the death of the cell, and the release of thousands of viruses.

The viral life cycle starts at the surface of the cell. Viruses float around randomly until they bump into a cell and find a particular receptor molecule on its surface. Poliovirus looks for an antibody-like receptor and rhinovirus looks for sialic acid carbohydrate attached to proteins on the cell surface. The viral capsid has a network of pockets that recognize these cellular receptors, attaching the virus to the surface of the cell. This triggers a change in the structure of the viral coat, causing it to inject the RNA through the membrane and into the cell. Once the RNA is inside, the cell's own ribosomes translate the RNA into a long polyprotein. This polyprotein is composed of all the viral proteins, strung together like a string of beads. The two proteases then cut themselves out of the polyprotein, and proceed to cut the rest of the proteins apart. Then, the major activity begins.

The new viral polymerase quickly begins making new copies of the viral RNA, using the cell's reservoir of nucleotides. A small viral protein called VPg is attached at one end, which then assists with further replication of viral RNA. One of the viral proteases seeks out a particular initiation factor used by the cell, and cuts it in half. This initiation factor is essential for protein synthesis with the cell's own messenger RNA, so all normal protein synthesis stops. But the viral RNA is designed so that it doesn't need this initiation factor to start protein synthesis, so the ribosomes devote all their energy to making viral proteins. As the number of viral RNA molecules rises and increasing numbers of capsid proteins are made from them, new viruses spontaneously begin to assemble. Each one contains a new RNA molecule packaged in a coat of newly built proteins. The viral polymerase and accessory proteins are left behind—there simply isn't enough room inside the capsid for anything but RNA. Finally, the cell ruptures and new viruses swarm out to infect other cells, and the cycle begins again.

Influenza Virus

Influenza has taken a devastating toll in human life. Influenza is a yearly danger, as the weather gets cold and our defenses weaken, and every few decades, a particularly effective strain appears and great pandemics of influenza sweep the globe. Some of these have been extremely deadly—for instance, over 40 million people died in the

pandemic of 1918–1919. The recurring effectiveness of influenza virus as a pathogen is a direct consequence of its genetic structure. Unlike the poliovirus and rhinovirus genomes, the influenza genome is carried in eight separate strands of RNA, each encoding different viral proteins. This segmented genome is the innovation that makes influenza so successful. It allows different strains of influenza virus to swap their RNA, mixing and matching different genes to create new strains that are more infectious and deadly.

New pathogenic strains of human influenza often develop with the help of other animals. There are a host of avian influenza viruses that attack birds. Typically, these viruses reproduce only in the digestive tract and do not cause disease in the birds, so the birds can act as a reservoir of viruses that lasts for years. Fortunately, these viruses rarely infect humans. But, they can easily infect pigs, and unfortunately, human influenza viruses also attack pigs. This is when the trouble occurs. If an avian virus infects a pig at the same time as a human virus, the two viruses can exchange RNA strands and create entirely new viruses, perhaps combining the most deadly features of both. This happens every decade or so, and a new strain appears. Since some of its genes came from a bird virus, nobody has immunity against it, so it can quickly spread from person to person.

The influenza virus is larger and more complex than poliovirus and rhinovirus. It is coated with a lipid membrane that surrounds the eight RNA strands. The membrane is studded with proteins that recognize the surfaces of cells that they infect. Differences in these proteins define the specificity of their targets: avian viruses can bind to bird cells (and pig cells), and human viruses can bind to human cells (and pig cells). The lipid membrane is not made by the virus: it is stolen from the cells that it infects. The last steps of assembly of the virus occur at the surface of the cell. New viruses are formed by budding, carrying with them a coating of cell membrane (Fig. 8.3).

Human Immunodeficiency Virus

The virus that causes AIDS (Fig. 8.1) is even more insidious than the viruses discussed above. Human immunodeficiency virus (HIV) is a retrovirus with a frightening difference in lifecycle. The virus carries two novel enzymes, a reverse transcriptase and an integrase, that together take over the cell. The virus injects its RNA strands into the cell (two identical copies) and the reverse transcriptase creates a piece



Fig. 8.3 Budding of Influenza Virus Influenza virus buds from the surface of cells instead of bursting out of cells like poliovirus. In this cross section, the interior of the cell is at bottom left and the budding virus fills most of the picture. The viral RNA, wrapped around protective proteins, forms large helical bundles inside the virus. Proteins lining the inside of the membrane power the budding process, and the proteins radiating outwards will recognize and bind to the cell this new virus will infect (1,000,000 X)

of DNA using the viral RNA as a template. Then, the integrase takes this piece of viral DNA and splices it into the cell's normal DNA.

This has a terrible consequence. Once the viral DNA is spliced into the DNA, it is virtually indistinguishable from the normal genes. It is

duplicated along with the genome when the cell divides. It is protected by the normal mechanisms that search out and repair damaged DNA. It may lie silent for years or decades after the initial infection. Because the virus is hidden in the genome, it is supremely difficult to combat. It must be recognized and destroyed before it enters a cell, for it is virtually invisible once it gets inside and splices itself into the cellular DNA.

Soon after infection with HIV, a vigorous battle ensues. The viruses preferentially infect cells in the immune system, forcing them to make many new viruses and ultimately killing them. The immune system fights back by building new white blood cells, destroying virus particles, and destroying infected cells. The battle continues for years, immune system against virus, but the immune system is slowly depleted in the process. As the levels of immune cells diminish, they become unable to fight other infections, such as pneumonia or tuberculosis, and the infected person begins to show the symptoms of AIDS.

The drugs used to combat infection by HIV attack the virus at all steps of its lifecycle (Fig. 8.4). The first drugs discovered to fight AIDS, such as azidothymidine (AZT), attack the reverse transcriptase, blocking the synthesis of the DNA before it can integrate into the infected cell's genome. New drugs are being developed that block other steps of infection, such as drugs that block the viral proteins that recognize cell surfaces, and other drugs that block the integrase enzyme that splices the viral DNA into the genome. Protease inhibitors such as ritonavir and indinavir attack the virus at the end of the lifecycle, as viruses are formed from an infected cell. They block a protein-cutting enzyme that clips viral proteins into the proper pieces as the virus buds from the cell surface and forms a mature, infectious virus. These drugs can keep the virus in check, but if the drugs are ever stopped, viruses that are integrated into the genome quickly pop up and cause a new round of infections. Also, as described in the next chapter, the virus mutates rapidly, giving the virus an effective way of fighting these drugs.

Vaccines

The polio vaccine ranks among the greatest triumphs of modern medicine, whereas the lack of a cure for the common cold is an oft-voiced complaint against medical research. The reason for this striking contrast is simple, and is based on an intrinsic difference between these two similar viruses. There are three major strains of poliovirus, each

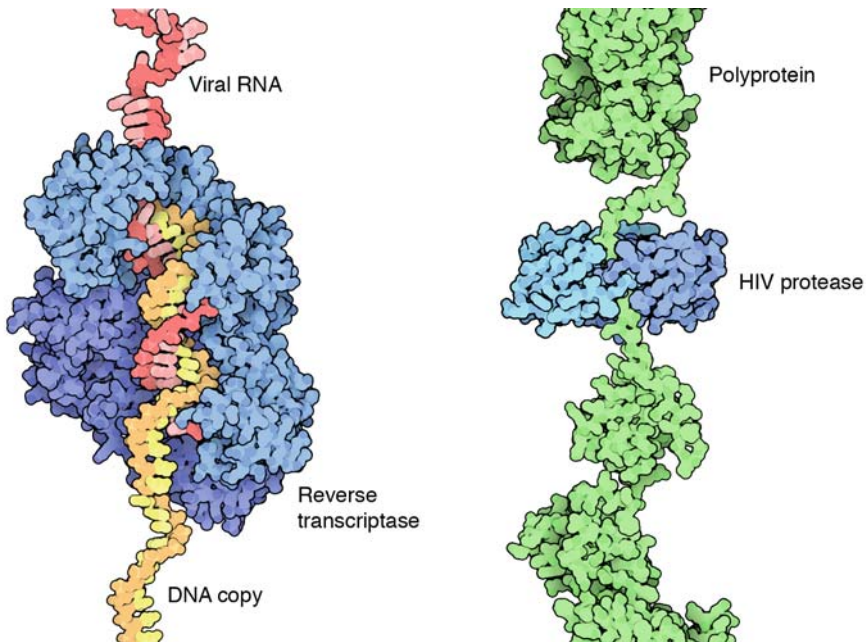


Fig. 8.4 HIV Enzymes Most of the drugs used to fight HIV infections block these two enzymes. On the left, reverse transcriptase is shown transcribing the viral RNA into a DNA copy. The enzyme destroys the RNA copy as it goes. On the right, HIV protease is cleaving the viral polyprotein into functional proteins (5,000,000 X)

with slightly different coat proteins. So if our body develops antibodies to destroy these three strains, it can control any polio infection it may encounter. Over a hundred strains of rhinovirus, however, are known to cause colds, along with dozens of other types of viruses. Each new cold that we catch is due to a strain of virus that we have never encountered before, and it is not practical to make a vaccine that protects against all of them.

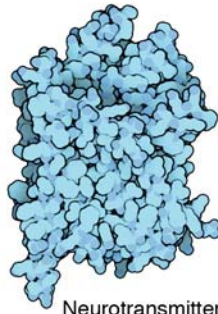
Vaccines prepare the immune system to fight future infections. When we are infected by a virus, the immune system mounts a defense against it, creating antibodies to recognize it and white blood cells to engulf and destroy it. Viruses are deadly when the immune system cannot react fast enough and the viruses multiply more quickly than they can be destroyed. The trick of vaccination is to challenge the immune system before an infection with a virus-like particle, so that we make all the necessary defenses without the danger of the real virus. Then, the immune system is primed and ready in case there is a real infection.

The most effective vaccines are composed of actual viruses, inactivated or weakened by chemical means, but still able to stimulate the body into making the proper defenses. The first polio vaccines were created by Salk and Youngner by inactivating purified poliovirus with formaldehyde. A few years later, an even more effective vaccine was developed by Albert Sabin. It contains live viruses, but viruses that have been mutated by forcing them to grow in non-human cells at lowered temperatures. These weakened viruses multiply for a short while in the gut, stimulating the immune system to make anti-poliovirus antibodies, but they do not infect nerve cells, so they don't cause poliomyelitis. The polio vaccine is particularly effective because all three strains can be included in these vaccines, so a polio vaccination protects against all three. Thanks to these vaccines, polio has been largely eradicated from the world.

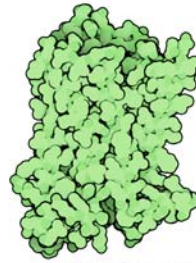
With our yearly vaccinations against influenza, however, we must play the odds. There are many, many strains of influenza virus circulating in the world, and more develop each year as the viruses exchange genes and mutate. Each year experts at institutions like the Centers for Disease Control examine the prevalence of certain strains worldwide and make a prediction about which strains pose the greatest danger in the coming year. Then the vaccine is made to protect against those strains. Two types of influenza vaccine are currently in widespread use: flu shots are an injection of inactivated viruses, and the nasal-spray vaccine is a live but weakened version of the influenza virus.

A vaccine to prevent infection by HIV is greatly needed, but still poses severe challenges. HIV is difficult to fight for many reasons. Because its reverse transcriptase makes lots of errors, HIV mutates very rapidly, so resistant strains rapidly develop when the virus is faced by any treatment. The virus is also resistant to the antibodies that normally provide protection because the proteins on the viral surface are coated with non-descript polysaccharides and the unique binding sites are hidden away in grooves too small for antibodies to reach. An HIV vaccine also needs to stimulate the immune system to respond more quickly and efficiently than usual. It only takes a week for HIV to integrate into the cell's genome, and after it does that, the integrated HIV is invisible to the immune system. HIV is also very difficult to study, since the similar viruses that attack monkeys and cats have significant differences, so many of the principles that are learned through study of them do not apply with HIV infection. For example, an effective vaccine that protects monkeys against infection by SIV (simian

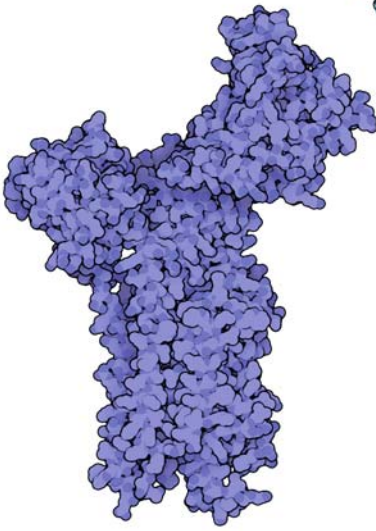
immunodeficiency virus) was discovered in the 1990s, but a similar vaccine does not work for infection of people by HIV. In spite of these challenges, many creative approaches are currently being tested to fight this worldwide danger to human health.



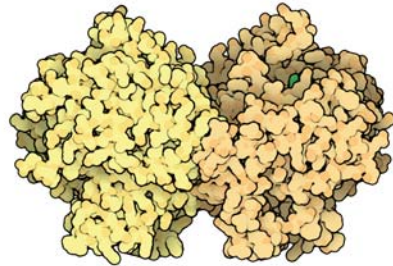
Neurotransmitter transporter



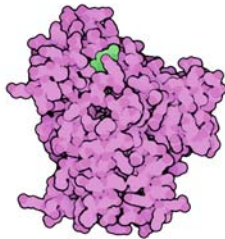
Neurotransmitter receptor



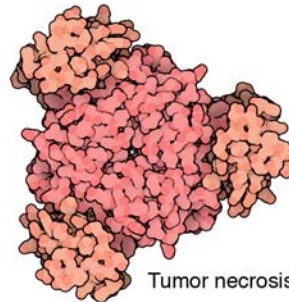
Proton Pump



Cyclooxygenase



Phosphodiesterase



Tumor necrosis factor

Chapter 9

You and Your Molecules

Your molecular machines are far too small to see. You might think that it would be impossible to affect them yourself, to speed them up or stop them, since they are so tiny and inaccessible. However, we modify the action of our own molecular machines every day. If you take a vitamin each morning, you are tuning up your molecular machines, making sure they are in top form. If your doctor gives you penicillin, you're actively attacking the molecular machines of the bacteria in an infection. If you are unlucky and get food poisoning, a bacterium has fought back, attacking one of your molecular machines. If you take an aspirin, you are blunting the function of molecular machines in your nerves and brain. With vitamins, poisons, and drugs we deliberately modify the action of specific machines, and by careful use, we can improve their action and thus our own quality of life (Fig. 9.1).



Fig. 9.1 You and Your Molecules Today, we have hundreds of drugs for modifying the action of our own molecular machines. A few common targets are shown here. Drugs that block the uptake of neurotransmitters by transporters in nerve cells are used to treat depression, and drugs that block different neurotransmitter receptors are used for a bewildering variety of ailments ranging from asthma to schizophrenia. Several forms of cyclooxygenase are the target for aspirin, both for fighting headaches and thinning blood. Tumor necrosis factor is targeted in therapy for rheumatoid arthritis and other inflammatory disorders. There are also a variety of “lifestyle” drugs becoming available, including drugs that block phosphodiesterase in blood vessels to induce erections, and drugs that block proton pumps and reduce stomach acid (5,000,000 X)

Vitamins

Vitamins are vital links in our metabolism. Ironically, they are molecules that we require for optimal health, but we cannot make them for ourselves. Bacteria are often far more self-sufficient than this. They can start with a few simple materials and build every molecule that they need. But somewhere in the evolution of human cells, we lost the ability to make a few essential molecules, presumably because they are always available in our diet. So today, we have no choice—we must obtain these important molecules in our food.

A rich body of folklore prescribes certain foods for specific ailments. For instance, carrots or liver will cure night blindness, cod liver oil will prevent rickets, and rose hip tea or citrus fruits will protect us from scurvy. Often, these folk cures are effective because of the vitamin content of the foods. Scientific analysis of these healing foods has revealed that specific molecules in each are responsible for the cure. The first one that was characterized, thiamine, gave vitamins their name: amines that are vital for life.

Many vitamins are molecular tools with unusual chemical characteristics, used by enzymes for specialized tasks. For instance, amino acids are all colorless, so in order to build a protein that senses light, a colored molecule is needed. Vitamin A, also known as retinal, is the perfect candidate for this job. Retinal is composed of a string of carbon atoms that readily absorbs the energy from a photon of light. But more importantly, when it absorbs the light energy, it changes shape from bent to straight (Fig. 9.2). This change in shape is easily sensed by the protein rhodopsin, which launches a nerve signal telling the brain that it has just captured a photon. We can obtain retinal directly in our diet in liver, but carrots and other colored vegetables contain molecules that are just as good as retinal. These molecules are bright yellow and orange carotenes, which are broken in half by enzymes in our cells to create molecules of retinal.

B vitamins are used to build special carrier molecules that shuttle hydrogen, nitrogen, or carbon atoms between various enzymes (Fig. 9.3). These molecules typically have a very reactive atom at one end, which performs a reaction that is too difficult for the amino acids in proteins. Thiamine (vitamin B1) has a reactive carbon atom that is needed to extract carbon dioxide out of food molecules, riboflavin (vitamin B2) and niacin are needed to make the two molecules that shuttle hydrogen and electrons around the cell, and

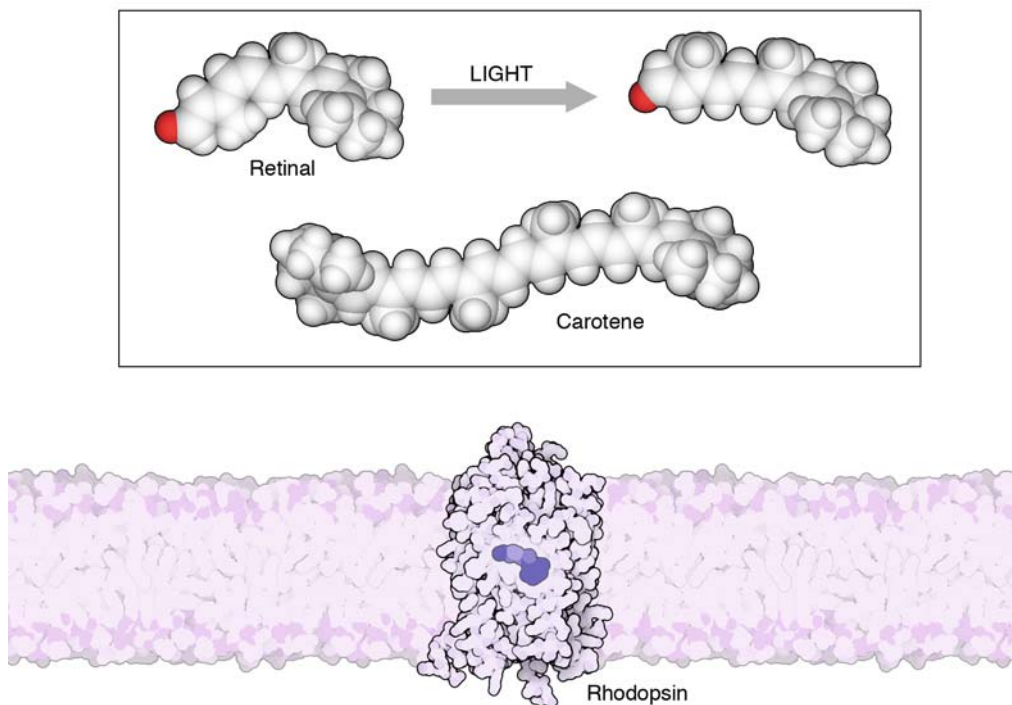


Fig. 9.2 Vitamin A Retinal, which is made from vitamin A, is used by the protein rhodopsin in our retinal cells to sense light. It normally adopts a bent conformation (as shown on the left), but when it absorbs a photon, it shifts to a straight form. Retinal can be obtained directly in the diet, or we can make it by breaking carotene in half (top, 20,000,000 X; bottom, 5,000,000 X)

pyridoxine (vitamin B6) has a special carbon atom that performs reactions that move nitrogen from place to place.

Vitamin C, also known as ascorbic acid, has a simple structure that belies its status as the most controversial of vitamins. In its most visible role, it is required by an enzyme that modifies the structure of new collagen molecules. If it is missing, the faulty collagen leads to the ravages of scurvy: loss of teeth, slow healing, and hemorrhaging. But this role requires only a small amount, making it hard to explain the large concentrations of vitamin C found in both animal and plant tissues. Vitamin C, along with vitamins A and E, also moonlights with a second job in the cell, acting as an antioxidant. As described in Chapter 7, this plays an essential role in controlling the inevitable aging of our cells and molecules.

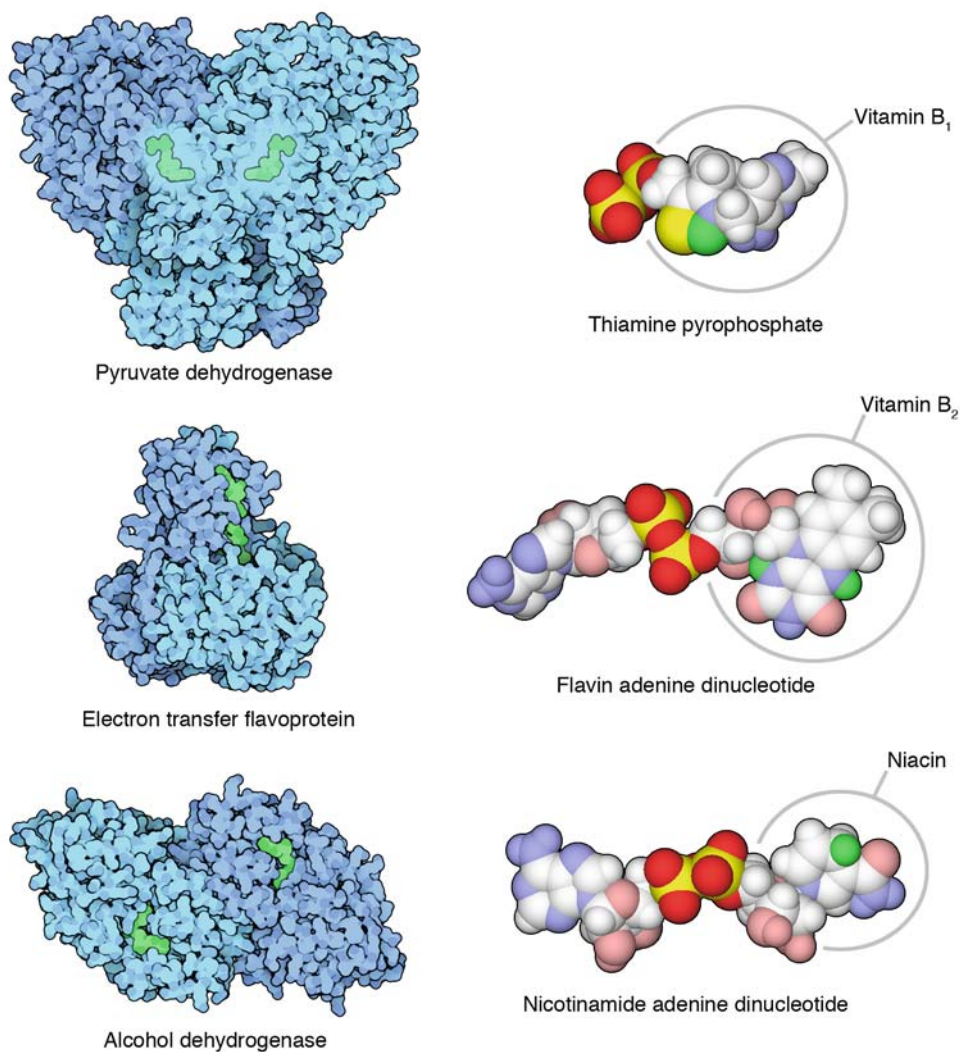


Fig. 9.3 B Vitamins B vitamins are used to build chemical tools used by enzymes. In the three shown here, the portion that is obtained as a vitamin the diet is circled in the close-up images, and examples of the enzymes that use these vitamins are shown on the left. Thiamine pyrophosphate is used by the enzyme pyruvate dehydrogenase to perform a tricky chemical reaction that involves the removal of carbon dioxide. A particularly reactive carbon atom, shown in green, assists in the reaction. Flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD) are used to carry electrons and hydrogen atoms (green). The electron transfer flavo-protein uses FAD to shuttle them between enzymes involved in fat metabolism, and alcohol dehydrogenase uses NAD in reactions that destroy (or in bacteria, to create) alcohol (left, 5,000,000 X; right, 20,000,000 X)

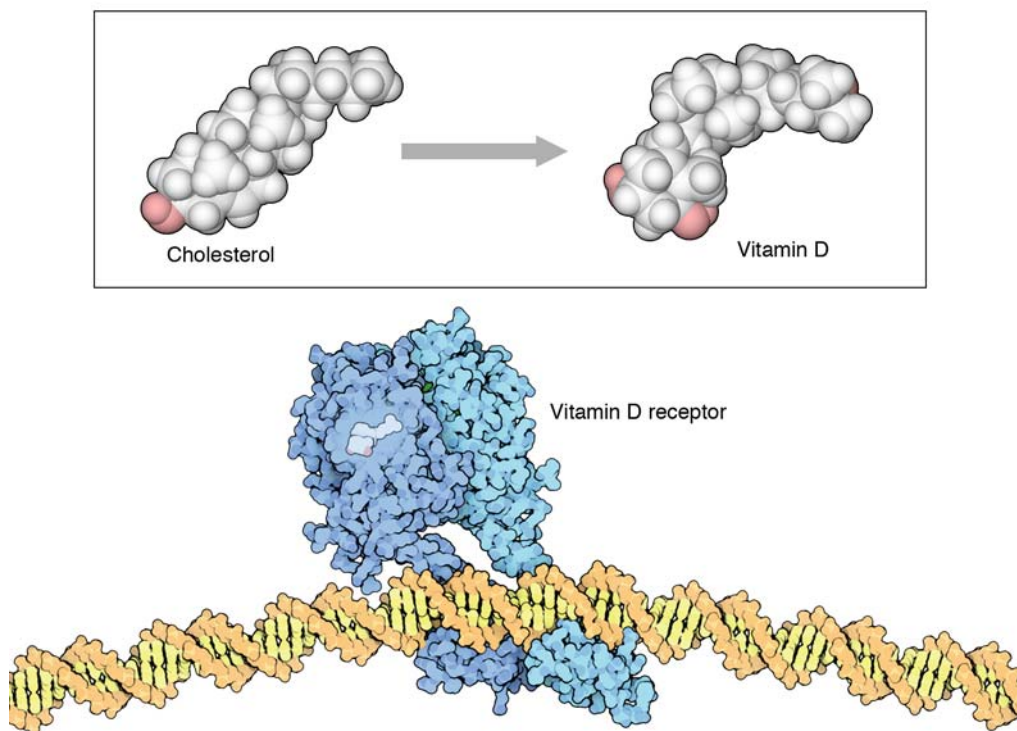


Fig. 9.4 Vitamin D Vitamin D is formed from cholesterol in a reaction that requires ultraviolet light. It acts as a hormone by entering cells and binding to receptors in the nucleus. These receptors bind to DNA and direct the formation of proteins involved in calcium metabolism (top, 20,000,000 X; bottom, 5,000,000 X)

Vitamin D plays a special role as a hormone that regulates the uptake and release of calcium in our bones (Fig. 9.4). Vitamin D stretches the definition of a vitamin. It is made directly by our cells, but only if we are exposed to enough sunlight. In our skin, ultraviolet light makes vitamin D by breaking a bond in cholesterol. Problems occur, however, when people cannot get into the sun, particularly people who live in high, cloudy latitudes. They cannot make enough of the vitamin themselves, and must take supplements such as the infamous doses of cod liver oil forced upon English children.

Broad-Spectrum Poisons

Our molecular machines are delicate. In the normal environment of the cell, they perform their jobs quickly and efficiently, without interference from the many other molecules in the cell. However, it is fairly easy to throw a monkey wrench into this system. If you add a molecule that binds tightly to a protein or nucleic acid, it can block its action. This is exactly what poisons do, and if the poison binds more tightly than the normal molecules in the cell, and attacks a protein that is particularly important, it can be deadly.

Some of the most effective poisons are also the smallest ones. Simple poisons that block the central process of energy production will kill almost any organism they contact. Since most modern organisms use virtually identical molecular machines to transform sugar into usable energy, a poison that blocks energy production in bacteria will be just as effective in plants and animals. These poisons are often chemically simple and easy to make, hence they have played a colorful role in human history.

Cyanide is one of the most effective poisons known. In minutes, cyanide will kill any organism that uses oxygen. Cyanide attacks the last step of energy production, when hydrogen atoms are added to oxygen to form water. The enzyme complex that performs this reaction—cytochrome c oxidase—contains an iron atom held in a heme group. Cyanide is about the same size and shape as oxygen, but it binds more tightly to the iron, blocking entry of oxygen and halting the reaction (Fig. 9.5). The cell suffocates, surrounded by oxygen but unable to use it. Cyanide can be found in nature in peach and apricot pits—a lethal dose can be obtained from about 100 g of ground pits. The pits contain amygdalen, marketed as Laetrile for cancer treatment, which releases cyanide in alkaline conditions such as those encountered in the intestines.

Carbon monoxide is similar in shape to oxygen and cyanide, and also binds tightly to iron atoms. The major site of carbon monoxide poisoning is the blood. It binds 250 times more tightly than oxygen to the iron atoms in hemoglobin, blocking the flow of oxygen from our lungs to cells throughout the body. Since it binds so tightly, carbon monoxide is difficult to remove from poisoned blood. An hour of breathing pure oxygen will reduce the level of bound carbon monoxide by only one half.

The environment is filled with many other poisonous compounds, not as instantly lethal as cyanide and carbon monoxide, but still a danger to our cells. Our food is filled with poisonous compounds made by plants to protect themselves. Cooking creates all sorts of reactive

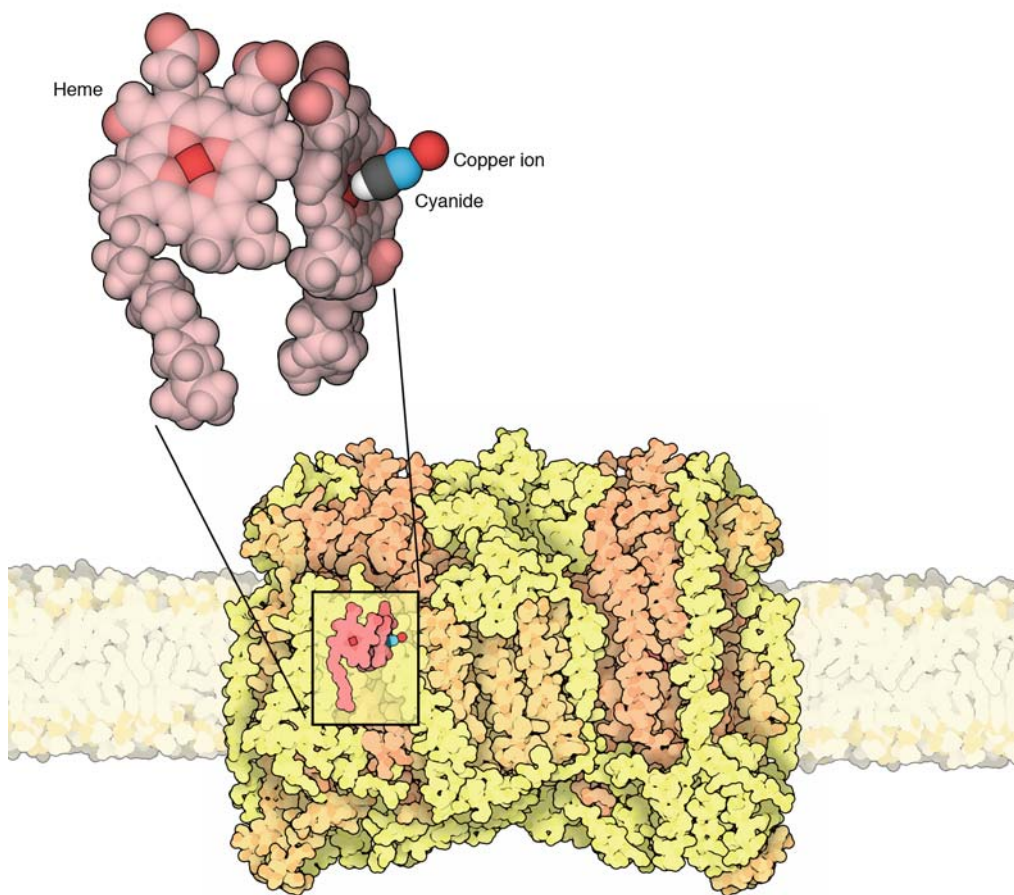


Fig. 9.5 Cyanide Cyanide ions are chemically similar to oxygen, but bind more tightly to enzymes that use oxygen. Often, a single molecule can halt the action of an entire protein. As shown here, cyanide binds to cytochrome c oxidase, the final protein in the process of respiration, blocking the use of oxygen (top, 20,000,000 X; bottom, 5,000,000 X)

compounds. Since we live in an industrial society, we breathe and drink all manner of pollutants. We also willfully consume poisonous compounds, such as the caffeine in coffee and the alcohol in wine and beer. Our molecular machines are delicate, and must be protected from these poisonous molecules. Fortunately, we have a powerful detoxification system that protects us from these dangers.

Cytochrome P450 is at the center of this detoxification system (Fig. 9.6). It is an enzyme that scoops up toxic compounds and attaches

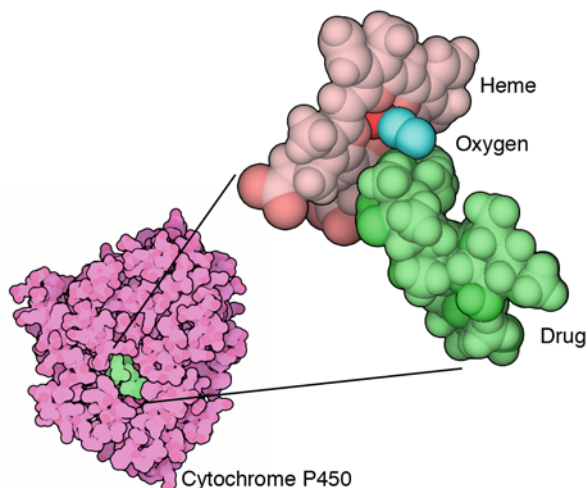


Fig. 9.6 Cytochrome P450 Cytochrome P450 enzymes add oxygen atoms to poisonous molecules. This makes them more soluble, and thus easier to flush out of the body, and also creates a convenient chemical handle for other detoxifying enzymes to take hold and destroy the poison. Cytochrome P450 enzymes use an iron atom held in a heme group to perform this chemical reaction. The one shown here, CYP3A4, performs much of the drug detoxification in the human body, destroying molecules such as acetaminophen (Tylenol), codeine, paclitaxel (Taxol), and several anti-HIV drugs. The green molecule is the drug erythromycin (left, 5,000,000 X; right, 20,000,000 X)

a chemical “handle” to them. Then, in a second step, other enzymes attach large, harmless chemical groups to the handle, making the poisonous molecule easy to recognize and flush out of the body. We make over a dozen different cytochrome P450 enzymes, each of which recognizes and detoxifies a different collection of poisonous molecules. If you have ever taken a painkiller, such as acetaminophen, you have seen cytochrome P450 in action. The effects of the painkiller wear off in a few hours as cytochrome P450 methodically destroys the molecules.

Bacterial Toxins

Small molecules like cyanide and carbon monoxide are highly poisonous, however, there are limits to how toxic they can be. Chemical poisons attack proteins one-on-one—one poison molecule attacking one protein—so as soon as they bind, they are finished. Pathogenic bacteria

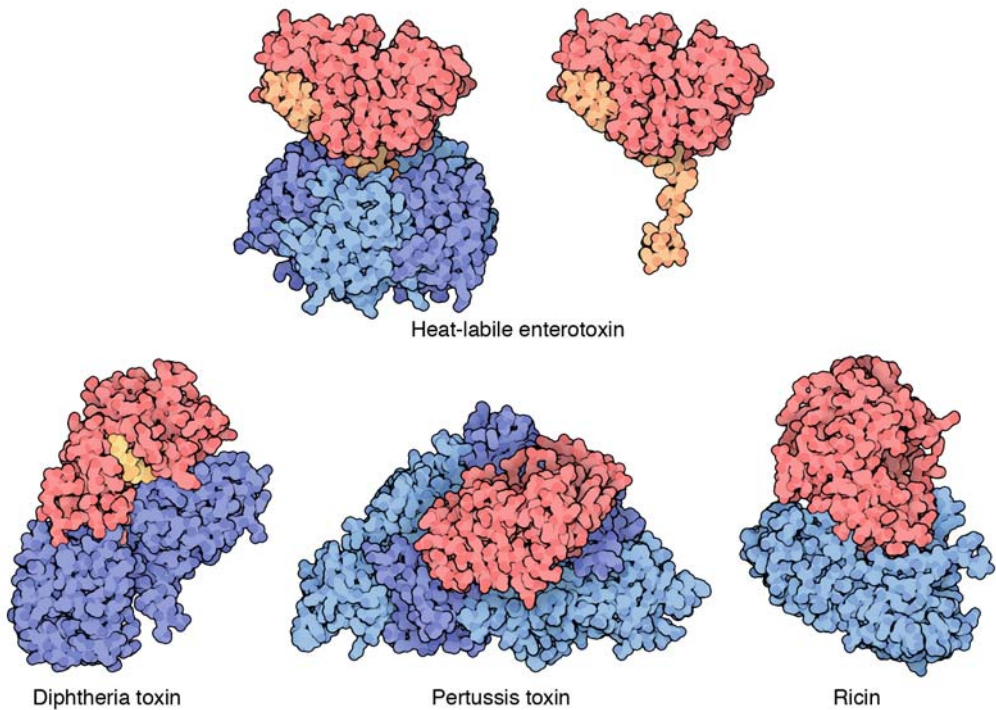


Fig. 9.7 Bacterial Toxins The two-part strategy for building toxins is used by many different organisms to protect themselves. In this picture, the targeting portion is colored blue and the toxic enzyme is colored red. The enterotoxin from *Escherichia coli*, which causes food poisoning and Montezuma's revenge, and pertussis toxin, the cause of whooping cough, both attack signaling pathways used by cells, ultimately scrambling the signals used to control flow of ions and water into the cell. The toxin made by diphtheria bacteria attacks one of the protein factors used in protein synthesis, and ricin, a powerfully lethal toxin made by the castor bean plant, uses the same strategy to attack ribosomes (5,000,000 X)

have devised an even more lethal approach. They use two biochemical strategies to make toxins that are extraordinarily deadly (Fig. 9.7). For example, a single molecule of diphtheria toxin, instead of simply disabling a single protein, can kill an entire cell.

The first strategy is to use a toxic enzyme instead of a simple chemical poison. Enzymes are catalysts that perform their reactions over and over again, hopping from target to target and making their chemical changes. So, a single toxic enzyme can disable a whole cell full of proteins, destroying one after the other until the cell is killed. This strategy is then combined

with a second strategy to make the toxin even more effective. The toxic enzyme is coupled with a targeting mechanism that finds cells and injects it directly inside. These targeting mechanisms often bind to polysaccharide chains on the surface of unlucky cells. Then they are drawn into the cells with normal food molecules. Once inside, they change shape and force the toxic enzyme into the cytoplasm, where it begins its destructive campaign.

If you have ever had food poisoning, you have probably been attacked by one of these terrible molecular machines. Bacteria like *Escherichia coli* build a toxin that attacks cells in the digestive system. The unpleasant symptoms of food poisoning occur as your body tries to flush the toxin out of your system. Other bacteria, such as the ones that cause cholera, diphtheria, and pertussis, build similar toxins, which can be even more deadly if not treated.

Antibiotic Drugs

We are lucky to live in a time when we are not completely helpless when faced by bacteria and their deadly toxins. Through decades of medical research, we have uncovered more and more information about bacteria and their molecules, and have discovered weaknesses that may be exploited to create bacteria-killing antibiotics. Today we have an arsenal of antibiotic drugs to fight infection by pathogenic bacteria.

Life-saving antibiotics and deadly poisons are two faces of the same coin. Poisons attack and destroy a key molecular machine, blocking one of the vital processes of life and killing cells. Antibiotics, on the other hand, are selective poisons, designed to attack in specific ways. Cyanide, of course, would be useless as an antibiotic drug, since it would kill both the pathogenic organism and the patient. Instead, antibiotic drugs must be designed to poison only an infecting micro-organism, without poisoning you at the same time.

It may seem strange to us now, but the concept that bacteria and viruses cause disease—that a physician's unwashed hands will infect a patient—has only been understood in the past century. Soon after the discovery of microscopic germs, the search began for specific compounds to poison them. The first approach, which is still used today, was to use an antiseptic. Antiseptics are mild poisons that kill micro-organisms by completely engulfing them. Since we only contact the antiseptic on our skin, only a few surface cells die, not our entire body. Solutions that contain chlorine, bromine, or iodine, as well as mercury compounds such as merthiolate, are effective disinfectants and are

often used to protect small wounds from infection. These reactive atoms attack sulfur compounds in bacterial enzymes and inactivate them. Alcohol is also widely used as a disinfectant. When engulfed in concentrated alcohol, bacterial proteins unfold and are inactivated.

The next great advance in medical biochemistry was the discovery of antibiotics that specifically attack molecular machines in pathogenic bacteria. Sulfanilamide was the first success. Since it attacks only a key bacterial enzyme used to make nucleotides, it may be taken internally to kill bacteria with minimal risk to the patient. Soon after, other antibiotics were discovered by looking at the natural ways that other organisms protect themselves from bacteria. The most familiar is penicillin, which is secreted by mold to control the growth of bacteria around them. Penicillin blocks the enzyme that builds the tough peptidoglycan layer that supports the bacterial cell wall (Fig. 9.8). When this enzyme is

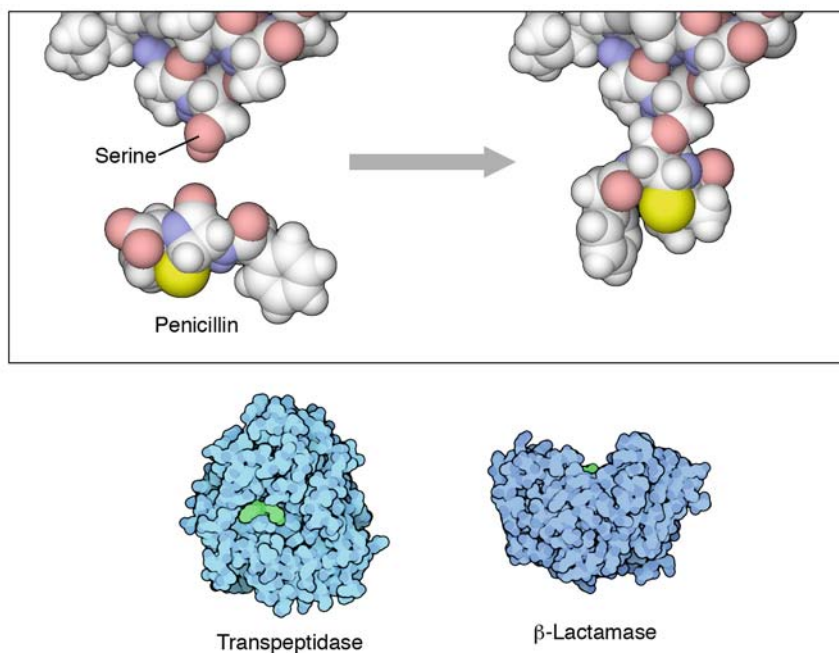


Fig. 9.8 Penicillin Penicillin is a spring-loaded trap that destroys a D-alanyl-D-alanine carboxypeptidase/transpeptidase, the enzyme that builds the peptidoglycan structure in bacterial cells. A chemically unstable ring of four atoms in penicillin attacks a serine amino acid in the protein, gluing the drug in the active site and blocking its action. Bacteria fight back, though, with proteins like β -lactamase, which destroys penicillin before it has a chance to act (top, 20,000,000 X; bottom, 5,000,000 X)

disabled, the cell is weakened and easily destroyed by the immune system. Since we do not build a similar peptidoglycan structure in our cells, the drug is completely safe and only attacks the bacteria.

Processes that are unique to pathogenic organisms provide the best targets for antibiotic action. Many examples have been found, and many more are under study now by medical researchers. Chloramphenicol and streptomycin attack bacterial ribosomes, which are smaller and structurally different than our own ribosomes, and rifampicin attacks the RNA polymerase in tuberculosis bacteria. Quinine interferes with disposal of waste products in the trypanosomes that cause malaria, which feed on hemoglobin from red blood cells and build up toxic levels of undigested heme. In recent years, the medical researchers have launched an enormous effort to find drugs that attack the unique enzymes of HIV, and now we have dozens of drugs that attack many steps of the viral lifecycle, including the enzymes reverse transcriptase, HIV protease, and integrase (as described in Chapter 8).

Unfortunately, the process of evolution is very fast in bacteria and viruses, because they reproduce so quickly. Drug-resistant strains of many bacteria have evolved in the decades since the discovery of antibiotics, and drug-resistant strains of HIV appear within weeks if the drug treatment is not carefully planned. This has led to a continual tug-of-war in modern medicine, with researchers developing new drugs, and bacteria and viruses evolving new ways to protect themselves from them.

Bacteria often gain resistance by making an enzyme that destroys the drug. For instance, some drug-resistant bacteria make a penicillin-cutting enzyme that is a modified form of a normal digestive enzyme (Fig. 9.8). It finds penicillin and breaks the key reactive bond, making it useless as a drug. The genetic instructions for making these drug-resistant enzymes is often encoded on small circles of DNA that are passed from bacterium to bacterium, spreading resistance through the population.

HIV becomes resistant to drugs in a different way. Instead of attacking the drug, drug-resistant strains of HIV have slight changes in the enzymes that are targeted by the drug (Fig. 9.9). The reverse transcriptase or protease enzymes normally attacked by the drug mutate so that the drug does not bind to it any more. HIV mutates very rapidly and can easily evade a single drug, developing resistance in a few weeks after the start of drug treatment. So the trick used in current HIV therapy is to treat infected people with a cocktail of several drugs at the same time. The different drugs attack entirely different targets, so the population of viruses is faced with the impossible task of mutating several molecular machines at the same time to evade all of the drugs.

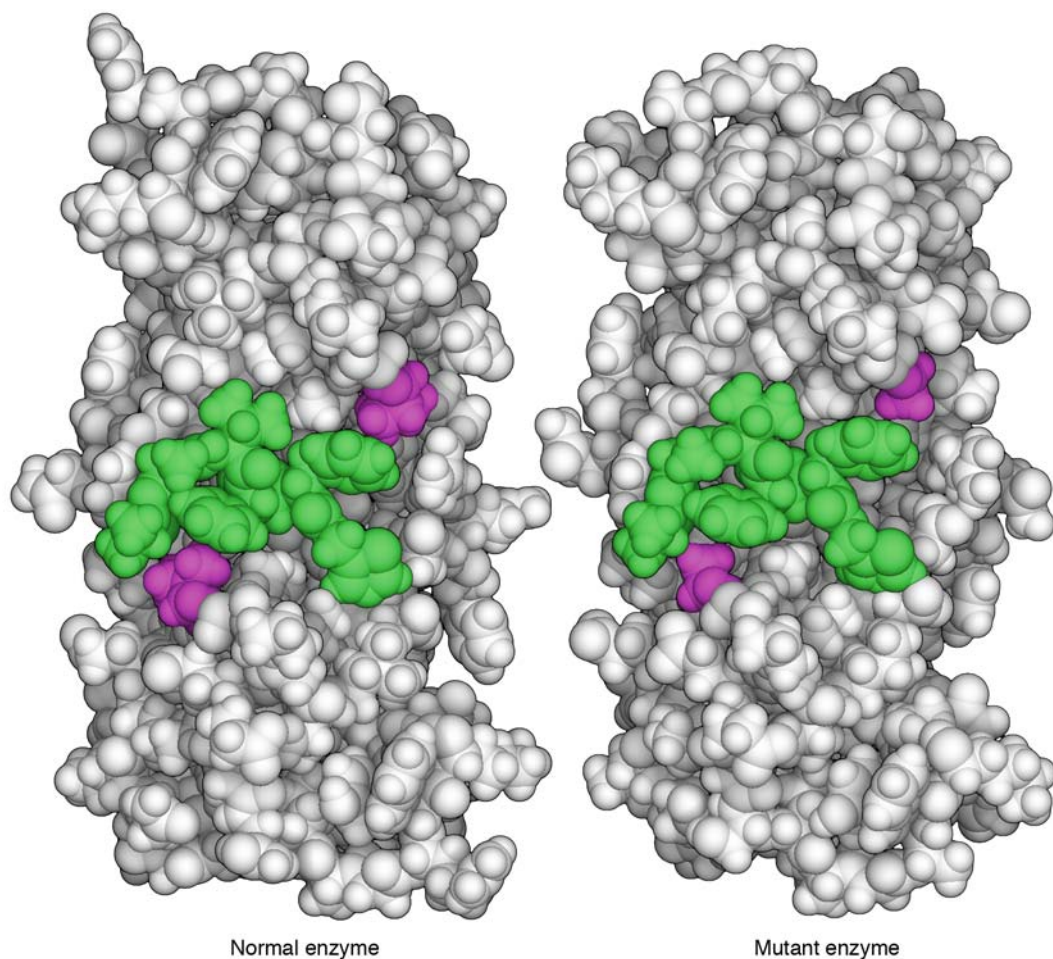


Fig. 9.9 Drug resistance in HIV In order for HIV drugs to be effective, they must bind tightly to HIV enzymes and block their action. HIV protease becomes resistant to drugs by mutating important amino acids in the active site. In this picture, the drug ritonavir is shown in green, and the amino acids at position 82 in the protein chains are shown in magenta. In the normal enzyme, the amino acid is a valine that forms close contact with the drug, stabilizing the binding. In the mutant enzyme, this amino acid has been changed to smaller alanine, weakening the contact just enough to make it ineffective as a drug (20,000,000 X)

Drugs and Poisons of the Nervous System

Medical practice from its very beginning has used specific chemical substances to make targeted changes to our own molecular machines. Willow bark and the resin from poppies were used to alleviate pain, foxglove was used in small quantities to treat heart conditions, and all manner of plants and mushrooms have been used to make changes to our sensory and nervous condition. In most of these cases, the plants are making these poisonous chemical compounds to protect themselves, and if we eat too much of them, they will cause serious problems. However, when used in carefully controlled doses, these chemicals are effective as medicines.

As our knowledge of the biochemistry of the human body has grown, we have gained the ability to modify these natural poison molecules and tailor them more closely to our needs. Today a wide variety of natural and synthetic drugs is available for gently modifying the action of our molecular machines, guiding them to perform in a more healthy way. These drugs are not designed to poison a molecular machine, like antibiotic drugs, but rather they are meant to slow the action of one of our own machines. In addition, drug therapy is most often temporary. Our natural detoxification machinery, such as cytochrome p450, destroys the circulating drugs within a few hours, so daily doses must be taken to give a consistent therapeutic result.

Many of these natural poisons and therapeutic drugs act on the nervous system. This makes perfect sense, since the nervous system is the central control of our body, and if we want to make adjustments, it is best to go to the source. Plants and fungi are especially adept at making poisons that attack the nervous system, since they have no nerves themselves. They are free to synthesize and store large quantities of these poisons with no ill effects, but any animals that eat them will be poisoned. Plants and fungi have been the source of many therapeutic compounds. A long tradition of folklore warns us away from poisonous plants and fungi, so we know to avoid eating deadly nightshade and toadstools. This same folklore, however, has shown that these poisons can be used in small, carefully controlled amounts to make beneficial changes to our nerves, relieving tension or blocking pain.

For example, curare, atropine, and hemlock paralyze muscles by blocking the transmission of signals between nerves and muscles. This signal is carried by acetylcholine, a small molecule that is released by the nerve cells and sensed by receptors on the surface of the muscle cell

(see Fig. 6.10). These poisons act by blocking the binding of acetylcholine to the receptor, so the muscle never gets the signal to contract. If you have ever had your eyes examined at the ophthalmologist, you have probably been poisoned by atropine. A small drop of the poison in each eye temporarily paralyzes the muscles that close the iris, dilating the pupils and allowing the doctor to see inside.

Strychnine acts in the opposite way. It blocks inhibitory receptors in nerve synapses that normally damp out the firing of nerves, so that nerves do not repeatedly stimulate themselves and the surrounding nerves. Strychnine blocks this control, with disastrous results. Instead of paralysis, victims of strychnine poisoning have uncontrollable convulsions. The slightest movement or sound will set off a chain reaction of nerve signals, contracting every muscle in the victim's body at the same time, stiffening the entire body into a rigid arc.

Depressants, such as diazepam (marketed as Valium), barbiturates, and alcohol, act in a more useful way at this inhibitory receptor. Instead of blocking inhibition and causing uncontrolled excitation of nerves, depressants are thought to facilitate the binding of neurotransmitters to inhibitory receptors, augmenting their effects and slowing the firing of nerves. The effects of different depressants are additive since they all act on the same protein, so alcohol will greatly enhance the effects of barbiturates. With increasing dose, the effects progressively mount as more and more nerves are drugged, starting with relief from anxiety and disinhibition, leading to sedation and sleep, and with toxic doses, to coma and death.

Other drugs act on specific classes of nerves, having more specific effects on thought and sense. For instance, painkillers block the signals that sense pain and carry the signal to the brain. Morphine and aspirin block these signals at opposite ends of the pain process. Aspirin blocks pain signals at their start. When damaged, cells in our skin and tissues release small molecules called prostaglandins, which then stimulate pain nerves to fire a signal to the brain. Aspirin blocks cyclooxygenase, one of the enzymes needed to make prostaglandins. Without the enzyme, there isn't enough prostaglandin available to send the pain signal. Morphine blocks pain at the other end, when the pain signal reaches the brain. It binds to receptors in the brain that normally recognize our own natural painkillers, the enkephalins. Enkephalins normally modify pain signals in the brain, blunting their edge and increasing our threshold for pain when needed by extreme circumstances. Since morphine co-opts this natural mechanism, it is the most potent painkiller known.

You and Your Molecules

Today, we have a surprisingly intimate relationship with our own molecules. We instinctively know to protect them from excessive heat and cold, from acid and alkali, from overly salty conditions, from strong sunlight. Our built-in sense of pain warns us that these dangerous conditions will inactivate them. We know from folklore that we need to keep them fed with vitamins and minerals to keep them in top shape, and we need to avoid poisonous compounds to keep them safe. Medicine has shown us that good hygiene will keep our cells clean and protected from pathogens like viruses and bacteria and has provided effective weapons to fight them if they get past our defenses. With these tools, we are living longer and healthier than any time in human history.

We are currently in the middle of a revolution in the understanding of our own bodies, as we explore the innermost secrets of our own molecules. We now understand the atomic details of most of the major molecular machines that orchestrate the nanoscale tasks of life. We are coming to understand when and where and how these machines are built, under the control of a resilient system of nanoscale information storage where errors are actually turned into evolutionary advantages. We are beginning to understand the seemingly paradoxical combination of order and chaos within the inner environment of the cell, in that remarkable realm where molecules become life.

With this understanding comes control. Today, medicine provides hundreds of ways that we can directly affect our own molecules for optimal health. But with understanding, we are also faced with new levels of responsibility. Every day, we are faced with dozens of decisions that require knowledge of our own molecules. We have to choose whether to eat a rich, fatty meal and take the chance of buildup of fat in our arteries. We have to decide if artificial steroids are unsportsmanlike. We have to choose whether or not to eat genetically modified produce or support research into gene therapy treatments for diabetes or cancer. Fortunately, as never before, we can base these choices on a detailed understanding of our bodies, from the molecular level up.

Atomic Coordinates

Atomic coordinates for the molecular illustrations in this book were taken from the RCSB Protein Data Bank. Coordinates and full references for each structure are available at the RCSB PDB WWW site at <http://www.pdb.org>. The accession codes are given below.

- 1.2. Glyceraldehyde-3-phosphate dehydrogenase 1gad, 1nbo, 3gpd
- 1.3. Hemoglobin 2hhb
- 2.1. ATP synthase 1c17, 1e79, 1l2p, 2a7u
- 2.2. Enolase 4enl
- 2.6. Transfer RNA 1ttt; ribosome 1yl4
- 2.7. Lysozyme 2lyz
- 2.8. Multidrug transporter 2onj; Rhodopsin 1f88; Insulin 2hiu; Glucagon 1gcg; Pepsin 5pep; Antibody 1igt; DNA polymerase 1tau; Ferritin 1hrs; ATP synthase 1c17; Collagen 1bkv; Actin 1atn
- 2.9. Lipid bilayer coordinates were obtained from the WWW site: <http://www.umass.edu/microbio/rasmol/bilayers.htm> (Heller, H., Schaefer, M. and Schulten, K. (1993) "Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid crystal phases" J. Phys. Chem. 97, 8343–8360.)
- 3.1. mRNA and tRNA 2j00
- 3.2. HMG-CoA reductase 1hwk; Oxidosqualene cyclase 1w6k
- 3.3. RNA polymerase 2e2i
- 3.4. Ribosome 1yl3, 1yl4; tRNA/EFTu 1ttt; EFG 1dar; phenylalanyl-tRNA synthetase 1ciy
- 3.5. Insulin 2hiu
- 3.6. Photosystem I 1jb0; Ribulose biphosphate carboxylase/oxygenase 1rcx
- 3.8. Aspartyl-tRNA synthetase 1c0a

- 3.9. Photosystem I 1jb0; Ferredoxin 1bxu; Cytochrome bc1 complex 1bgy; Plastocyanin 1bgu; ATP synthase 1c17, 1e79, 1l2p, 2a7u
- 3.10. Vitamin B12 transporter 2qi9; Sodium/proton antiporter 1zcd; Magnesium transporter 2bbj
- 7.2. Ubiquitin 1f9j; Proteasome 1fnt
- 7.3. DNA with a thymine dimer 1ttd; DNA photolyase 1tez
- 7.4. Rad51 1szp
- 7.5. Caspase-9 1nw9; Caspase-3 1pau; Caspase-activated deoxyribonuclease 1v0d, 1c9f
- 7.6. Src 2src
- 7.7. Cytochrome c oxidase 1oco
- 7.8. Superoxide dismutase 2sod; Catalase 8cat; Peroxiredoxin 2pn8
- 7.9. Ascorbate 1oaf; Cytochrome b₅ reductase 1ib0
- 8.4. Reverse transcriptase 1jlb; HIV protease 1hsg
- 9.1. Proton pump 1su4; Neurotransmitter transporter 2qju; Neurotransmitter receptor 2rh1; Cyclooxygenase 1prh; Tumor necrosis factor 1tnr; Phosphodiesterase 1udt
- 9.2. Rhodopsin 1f88
- 9.3. Pyruvate dehydrogenase 1ni4; Electron transfer flavoprotein 1efv; Alcohol dehydrogenase 2ohx
- 9.4. Vitamin D receptor 1db1, 1kb6
- 9.5. Cytochrome c oxidase 1oco
- 9.6. Cytochrome P450 1j0d
- 9.7. Heat labile enterotoxin 1lts; Diphtheria toxin 1mdt; Pertussis toxin 1prt; ricin 2aai
- 9.8. Transpeptidase 1hvb; beta-Lactamase 4blm
- 9.9. HIV protease 1hxw, 1rl8

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